

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

Jackson MR. **Priorities in the development of alternative methodologies in the pharmaceutical industry.** Arch Toxicol Suppl 1998;20:61-70.

Promotion of animal welfare is an underlying and laudable goal for toxicologists and there is good reason to adopt practical, focused, investigative approaches towards this aim. Pharmaceutical regulatory toxicology can be subdivided into the areas of systemic (target organ), reproductive, genetic and topical toxicology, as well as immunotoxicology and oncology. These areas can be assessed for prioritisation as to where adoption of measures to promote any or all of the 3 Rs (reduction, replacement, refinement) would lead to the most tangible benefit for animals. These measures can range, for example, from replacement of animal experimentation with alternative in vitro techniques, to adoption of regulatory protocols that reduce the number of animals required. This paper is confined to consideration of in vitro technology in terms of reducing/replacing laboratory animal use, and a suggested list of criteria for prioritisation is potential for:- Regulatory acceptability Reducing development cost Reducing animal numbers Promoting welfare aspects Elucidating toxic mechanisms Usefulness in compound selection Advancing the science of toxicology Clear messages emerge from such an analysis which could influence prioritisation of the application of in vitro toxicology from the standpoints of animal welfare, feasibility and resources.

Krysiak B, Rydzynski K. **[The comparative studies of the usefulness of the fixed dose procedure and the up-down method in the evaluation of acute chemical toxicity]**. Med Pr 1997;48(5):561-78. (Pol) BIOSIS COPYRIGHT: BIOL ABS. Following the studies on four compounds, classified in three different groups of toxicity, the validity of the up-down method and the fixed dose procedure was compared to classic Litchfield and Wilcoxon tests. The study was based rather on a very careful clinical observation than on the estimation of animal mortality as recommended in DL50 test. The study showed a considerable consistence between classic tests and the up-down method and the fixed dose procedure. But the determination of acute toxicity of chemical compounds by means of alternative methods proved to be highly humanitarian, and it required much lower number of less suffering animals.

Levin BC. **New approaches to toxicity: a seven-gas predictive model and toxicant suppressants.** Drug Chem Toxicol 1997;20(4):271-80.

Two new research approaches in combustion toxicology are: 1. the prediction of smoke toxicity from mathematical equations, which are empirically derived from, experiments on the toxicological

interactions of complex fire gas mixtures and 2. the use of toxicant suppressants in materials or products to prevent the formation of toxic combustion products. The predictive approach consists of burning materials using a bench-scale method that simulates realistic fire conditions, measuring the concentrations of the primary fire gases--CO, CO₂, low O₂, HCN, HCl, HBr, and NO₂--and predicting the toxicity of the smoke using either the 6- or 7-gas N-Gas Model. These models are based on the results of toxicological studies of these primary gases as individual gases and as complex mixtures. The predicted toxic potency is checked with a small number of animal (Fischer 344 male rats) tests to assure that an unanticipated toxic gas is not generated or an unexpected synergistic or antagonistic effect has not occurred. The results indicate if the smoke from a material or product is extremely toxic (based on mass consumed at the predicted toxic level) or unusually toxic (based on the gases deemed responsible). The predictions based on bench-scale laboratory tests have been validated with full-scale room burns of a limited number of materials of widely differing characteristics chosen to challenge the system. The advantages of this new approach are 1. the number of test animals is minimized by predicting the toxic potency from the chemical analysis of the smoke, 2. smoke may be produced under conditions that simulate the fire scenario of concern, 3. fewer tests are needed, thereby reducing the overall cost of the testing and 4, information is obtained on both the toxic potency of the smoke and the responsible gases. The N-Gas Models have been developed into the N-Gas Method (described in this paper) and these results have been used in computations of fire hazard. The 6-Gas Model is now part of the international standard ISO 13344 approved by 16 member countries of the International Standards Organization (ISO) and is also included in the U.S. national standard ASTM E1678 approved by the American Society for Testing and Materials (ASTM). In addition, the 6-Gas Model is used in the American National Standard--NFPA 269--approved by the National Fire Protection Association (Quincy, MA). The second new research approach, toxicant suppressants, examines the potential of chemical compounds, which when added to a material, to inhibit or reduce the concentration of a specific toxic gas normally generated during the material's thermal decomposition. The effectiveness of this approach was demonstrated at the National Institute of Standards and Technology (NIST) when HCN generation was reduced by 90% and the resultant toxicity of the combustion products was lowered by 50% when a flexible polyurethane foam (FPU) was treated with 0.1% (by weight) cuprous oxide (Cu₂O). Copper and cupric oxide (CuO) also reduced the HCN generation but were not as efficient as Cu₂O. Although melamine-treated FPU foams are being promoted as more fire safe than standard foams, a melamine-treated foam generated 10 times more HCN than a foam without melamine. The addition of Cu₂O to this melamine foam also reduced the HCN generation by 90%.

Nalecz-Jawecki G, Sawicki J. **Toxicity of inorganic compounds in the Spirotox test: a miniaturized version of the Spirostomum ambiguum test.** Arch Environ Contam Toxicol 1998;34(1):1-5.

The Spirostomum ambiguum toxicity test has been intensively studied in the Department of Environmental Health Sciences, Warsaw University of Medicine for the last 5 years. The purpose of the present work was to develop and evaluate a miniaturized microplate version of the test, called the Spirotox test, and to estimate the toxicity of selected inorganic compounds to the Spirostomum ambiguum. The test was carried out in conventional 24-well (6 x 4) polystyrene multiwell plate. Preliminary test was one control and 11 toxicant concentrations with two duplicates. Definitive test was one control and five toxicant concentrations with three duplicates per concentration. Dilution of the sample was made directly in the plate. Toxicity series of heavy metals based on 24-h LC50 may be

established as follows: Cu > Ag > Hg > Cr > Cd > Zn > Ni > Pb > Co > Mn. The series may be divided into four classes: extremely toxic: below 0.1 ppm (Cu, Ag, Hg); very toxic: 0.1-1.0 ppm (Cr, Cd, Zn, Ni); toxic: 1.0-10 ppm (Pb, Co); and low toxic: above 10 ppm (Mn). Anions were much less toxic to S. ambigua than cations. Using the same classification, only cyanide (CN) was toxic, other anions were low toxic. Toxicity series based on 24-h LC50 may be established as follows: CN > SeO3 > Cr2O7 > NO2 > S2O3 > WO4 > BO3.

Purchase IF. Development and strategic use of alternative tests in assessing the hazard of chemicals. Arch Toxicol Suppl 1998;20:71-80.

Rucki M, Tichy M. **Acute toxicity of alcohols: prediction by QSAR analysis and by molecular similarity.** Cent Eur J Public Health 1997;5(4):183-7.

The purpose of the paper is to demonstrate the usefulness of techniques of molecular similarity as an alternative method for estimation of both acute toxicity of chemicals and their partition coefficients. The technique of atom pairs was chosen. A series of aliphatic alcohols was treated. Acute toxicity was determined by the express laboratory method as the EC50 (inhibition of movement of the worms *Tubifex tubifex*). Partition coefficients were determined between oil and gas. The calculated results were sufficiently close to both experimental results and the estimates calculated by a technique of QSAR analysis.

Sandoe P, Svendsen O. **Animal burdens versus human benefits--how should the ethical limits be drawn for use of animals as models in toxicology?** Arch Toxicol Suppl 1998;20:31-40.

CARCINOGENESIS

Ali RB, Teo AK, Oh HK, Chuang LS, Ayi TC, Li BF. **Implication of localization of human DNA repair enzyme O6-methylguanine-DNA methyltransferase at active transcription sites in transcription-repair coupling of the mutagenic O6-methylguanine lesion.** Mol Cell Biol 1998;18(3):1660-9.

DNA lesions that halt RNA polymerase during transcription are preferentially repaired by the nucleotide excision repair pathway. This transcription-coupled repair is initiated by the arrested RNA polymerase at the DNA lesion. However, the mutagenic O6-methylguanine (6MG) lesion which is bypassed by RNA polymerase is also preferentially repaired at the transcriptionally active DNA. We report here a plausible explanation for this observation: the human 6MG repair enzyme O6-methylguanine-DNA methyltransferase (MGMT) is present as speckles concentrated at active transcription sites (as revealed by polyclonal antibodies specific for its N and C termini). Upon treatment of cells with low dosages of N-methylnitrosourea, which produces 6MG lesions in the DNA, these speckles rapidly disappear, accompanied by the formation of active-site methylated MGMT (the repair product of 6MG by MGMT). The ability of MGMT to target itself to active transcription sites, thus providing an effective means of repairing 6MG lesions, possibly at transcriptionally active DNA, indicates its crucial role in human cancer and chemotherapy by alkylating agents.

Contrera JF, DeGeorge JJ. **In vivo transgenic bioassays and assessment of the carcinogenic potential of pharmaceuticals.** Environ Health Perspect 1998;106(Suppl 1):71-80.

There is general agreement in the scientific community on the need to improve carcinogenicity testing and the assessment of human carcinogenic risk and to incorporate more information on mechanisms and modes of action into the risk assessment process. Advances in molecular biology have identified a growing number of genes such as protooncogenes and tumor-suppressor genes that are highly conserved across species and are associated with a wide variety of human and animal cancers. In vivo transgenic rodent models incorporating such mechanisms are used to identify mechanisms involved in tumor formation and as selective tests for carcinogens. Transgenic methods can be considered an extension of genetic manipulation by selective breeding, which long has been employed in science and agriculture. The use of two rodent species in carcinogenicity testing is especially important for identifying transspecies carcinogens. The capacity of a substance to induce neoplasia across species suggests that the mechanism(s) involved in the induction of the neoplasia are conserved and therefore may have significance for humans. Based on available information there is sufficient experience with some in vivo transgenic rodent carcinogenicity models to support their application as complementary second species studies in conjunction with a single 2-year rodent carcinogenicity study. The optional substitution of a second 2-year rodent carcinogenicity study with an alternative study such as an in vivo transgenic carcinogenicity study is part of the International Conference on Harmonization guidance S1B: Testing for Carcinogenicity of Pharmaceuticals. This guidance is intended to be flexible enough to accommodate a wide range of possible carcinogenicity assessment models currently under consideration or models that may be developed in the future. The use of an in vivo transgenic mouse model in place of a second 2-year mouse study will improve the assessment of carcinogenic risk by contributing insights into the mechanisms of tumorigenesis and potential human relevance not available from a standard 2-year bioassay. It is envisioned that this will stimulate the further development of more efficient and relevant methods for identifying and assessing potential human carcinogenic risk, which will benefit public health.

Cunningham AR, Rosenkranz HS, Klopman G. **Structural analysis of a group of phytoestrogens for the presence of a 2-D geometric descriptor associated with non-genotoxic carcinogens and some estrogens.** Proc Soc Exp Biol Med 1998;217(3):288-92.

Analysis of a group of phytoestrogens indicates that the majority of these chemicals are devoid of a 6A distance descriptor biophore. This 6A biophore is associated with the carcinogenicity of a set of chemicals in the mouse subset of the Carcinogenic Potency Database assembled by Gold et al. The prevalence of non-DNA-reactive carcinogens and chemicals endowed with estrogenic activity included in the group of chemicals possessing 6A descriptor suggests that it describes a ligand binding site on an estrogen receptor. Evidence is presented that estrogens with and without the 6A biophore bind to alternate receptors or to similar receptors but with different affinities. Since the 6A biophore was identified based upon a carcinogenicity database, it is conceivable that binding to the putative receptor that recognizes this biophore is associated with carcinogenicity. Alternatively, estrogens devoid of this 6A biophore may be noncarcinogenic, suggesting that carcinogenicity and estrogenicity may be separate phenomena.

Cunningham AR, Rosenkranz HS, Zhang YP, Klopman G. **Identification of 'genotoxic' and 'non-**

genotoxic' alerts for cancer in mice: the carcinogenic potency database. *Mutat Res* 1998;398(1-2):1-17.

A set of chemicals tested for carcinogenicity in mice that have been analyzed by Gold et al. [L.S. Gold, C.B. Sawyer, R. Magaw, G.M. Backman, M. deVeciana, R. Levinson, N.K. Hooper, W.R. Havender, L. Bernstein, R. Peto, M.C. Pike, B.N. Ames, *Environ. Health Perspect.* 58 (1984) 9-319; L.S. Gold, M. deVeciana, G.M. Backman, M. Lopipero, M. Smith, R. Blumenthal, R. Levinson, L. Bernstein, B.N. Ames, *Environ. Health Perspect.* 67 (1986) 161-200; L.S. Gold, T.H. Slone, G.M. Backman, R. Magaw, M. DaCosta, P. Lopipero, M. Blumenthal, B.N. Ames, *Environ. Health Perspect.* 74 (1987) 237-329; L. S. Gold, T.H. Slone, G.M. Backman, S. Eisenberg, M. DaCosta, M. Wong, N.B. Manley, L. Rohrbach, B.N. Ames, *Environ. Health Perspect.* 84 (1990) 215-286; L.S. Gold, N.B. Manley, T.H. Slone, T.H. Garfinkle, L. Rohrbach, B.N. Ames, *Environ. Health Perspect.* 100 (1993) 65-135] in the first five plots of the carcinogenic potency database (CPDB) was subjected to CASE/MULTICASE analyses. Briefly, CASE/MULTICASE is a computer-automated structure evaluation system that is capable of identifying structural features of chemicals associated with a specified biological activity (e.g., carcinogenicity or mutagenicity). These features are then incorporated into a structure-activity relationship (SAR) model for the analyzed database. The mouse CPDB used in this study consists of 627 chemicals, 289 of which are carcinogens, 11 marginal or weak carcinogens (i.e., chemicals requiring high doses to induce cancer) and 327 non-carcinogens. In an internal prediction analysis where the CASE/MULTICASE SAR model was used to predict the carcinogenicity of chemicals used to create the model, a concordance between experimental and predicted results of 96% was obtained. This indicates that the model is able to satisfactorily explain the chemicals in the learning set. In a drop-one cross-validation study where chemicals were removed one at a time and the remaining $n - 1$ chemicals were used in an iterative method to create a model to predict the removed chemical, CASE/MULTICASE was able to achieve a concordance between experimental and predicted results of 70%. Using a modified validation process designed to investigate the predictivity of a more focused SAR model, the system achieved a 78% concordance between experimental and predicted results. Among the major biophores identified by CASE/MULTICASE associated with cancer causation in mice several are derived from electrophilic or potentially electrophilic compounds (e.g., hydrazines, N-mustards, N-nitrosamines, aromatic amines, reactive halogens, and quinones). Other biophores however are derived from chemicals seemingly devoid of actual or potential DNA-reactivity and as such may represent structural feature of non-genotoxic carcinogens.

DeGeorge JJ, Ahn CH, Andrews PA, Brower ME, Giorgio DW, Goheer MA, Lee-Ham DY, Mcguinn WD, Schmidt W, Sun CJ, et al. **Regulatory considerations for preclinical development of anticancer drugs.** *Cancer Chemother Pharmacol* 1998;41(3):173-85.

Dhalluin S, Elias Z, Cruciani V, Bessi H, Poirot O, Rast C, Gate L, Pages N, Tapiero H, Vasseur P, et al. **Two-stage exposure of Syrian-hamster-embryo cells to environmental carcinogens: superinduction of ornithine decarboxylase correlates with increase of morphological-transformation frequency.** *Int J Cancer* 1998;75(5):744-9.

As part of environmental toxicology, it is important to assess both the carcinogenic potential of xenobiotics and their mode of action on target cells. Since dysregulation of ornithine decarboxylase (ODC), a rate-limiting enzyme of polyamine biosynthesis, is considered as an early and essential

component in the process of multistage carcinogenesis, we have studied the mode of ODC induction in Syrian-hamster-embryo(SHE) cells stage-exposed to carcinogens and to non-carcinogens. One-stage (5 hr) treatment of SHE cells with 50 microM clofibrate (CLF), a non-genotoxic carcinogen, or with 0.4 microM benzo(a)pyrene (BaP), a genotoxic carcinogen, slightly decreased basal ODC activity. Using the 2-stage exposure, 1 hr to carcinogen, then replacement by TPA for 5 hr, the ODC activity was higher than that obtained with TPA alone. This ODC superinduction was not observed when SHE cells were similarly pre-treated with non-carcinogenic compounds. Several environmental chemicals, pesticides, solvents, oxidizers and drugs were investigated with this SHE cell model. With one-stage exposure, some xenobiotics decreased basal ODC activity, while for others ODC changes were not noticeable. With 2-stage exposure (chemical followed by TPA), all carcinogens amplified the TPA-inducing effect, resulting in ODC superinduction. Comparative studies of the action of carcinogens and of non-carcinogens, using 2-stage exposure protocols, clearly show a close relationship between ODC induction rate and morphological transformation frequency.

Eastin WC. **The U.S. National Toxicology Program evaluation of transgenic mice as predictive models for identifying carcinogens.** Environ Health Perspect 1998;106(Suppl 1):81-4.

National Institute of Environmental Health Sciences researchers have invested considerable effort in exploring the utility of transgenic mice to detect carcinogens and study mechanisms of carcinogenesis. Work has assessed several mouse models genetically altered to enhance their expression of chemically induced tumors. Results with the p53def (hemizygous for the tumor-suppressor gene) and the Tg.AC (carrier of an activated H-ras oncogene) mice have been used as a basis for a proposed new strategy for identifying chemical carcinogens and assessing risk. The U.S. National Toxicology Program is conducting a series of studies with these two transgenic strains to further examine their strengths and weaknesses for identification of documented rodent and human carcinogens and to explore their ability to provide information concerning the effective dosimetry for target organ mutation.

Ferris DG, Wright TC Jr, Litaker MS, Richart RM, Lorincz AT, Sun XW, Woodward L. **Comparison of two tests for detecting carcinogenic HPV in women with Papanicolaou smear reports of ASCUS and LSIL [see comments].** J Fam Pract 1998;46(2):136-41.

BACKGROUND: The detection of cancer-associated types of human papillomavirus (HPV) in cervical specimens predicts the presence and future development of cervical intraepithelial neoplasia (CIN). The purposes of this study were (1) to determine the efficacy of a second-generation assay by hybrid capture (HC II) to detect carcinogenic HPV from residual cervical cells of a liquid-based cervical cytologic specimen, and (2) to compare the performance of this second-generation test with the first-generation hybrid capture (HCT) HPV test of material from direct cervical sampling to detect CIN in women with atypical squamous cells of undetermined significance (ASCUS) and low-grade squamous intraepithelial lesion (LSIL) Papanicolaou (Pap) smear reports. **METHODS:** Women with a recent Pap smear report of ASCUS or LSIL had a sampling of the cervix using either an Ayre's spatula and cytobrush or an Accellon device sampling for liquid-based cytologic system HC II HPV testing, followed by a Dacron swab sampling of the cervix for standard HCT HPV testing of the paired specimens. All women received colposcopy examinations including cervical biopsy and endocervical curettage, when indicated, to determine criterion standards for comparison. **RESULTS:** Paired swabs and liquid-based cervical specimens from 242 women were available for testing by standard HCT and the newer HC II HPV DNA

assays. The sensitivity, specificity, and positive and negative predictive values for detecting CIN grade 2 or 3 (CIN 2/3) were 61.9%, 57.0%, 12.0%, and 94.0%, respectively, for the HCT test, and 90.5%, 29.4%, 10.9%, and 97.0%, respectively, for the liquid-based cytology HC II assay. When only women with an initial ASCUS Pap smear report were considered, the HC II test results were 88.9%, 40.3%, 9.1%, and 98.2%, respectively. **CONCLUSIONS:** Testing for lower genital tract carcinogenic HPV DNA using a cervical cytology liquid transport media residual sample is clinically feasible. The new HC II microplate HPV test achieved a greater test sensitivity for detecting carcinogenic HPV and correspondingly of CIN 2/3 compared with the currently available first-generation HC HPV test. Use of a liquid-based cervical cytology system combined with intermediate triage by HC II testing of residual cells for carcinogenic HPV alone may help to efficiently identify CIN 2/3 in women who have a prior screening Pap smear report of ASCUS.

Foa V, Elia G, Schiavulli N, Sartorelli P, Schiarra G, Cenni A, Novelli MT, Mangani F, Cecchetti G, Iachetta R. **[Non-bioavailability of carcinogenic polycyclic aromatic hydrocarbons contained in coal dust]**. *Med Lav* 1998;89(1):68-77. (Ita)

The aim of the study was to ascertain whether polycyclic aromatic hydrocarbons (PAHs) especially 3,4-benzo(a)pyrene, could be eluted from coal dust that comes into contact with biological fluids or crosses the skin barrier. A fine-grained coal dust with known PAH content was placed into contact with homogenized pig lung or human gastric juices, and also applied to monkey skin, using the cutaneous window method. The results demonstrate that, in the in vitro systems used, PAHs contained in coal dust are not eluted by lung homogenate or gastric juices, nor are they capable of crossing the skin barrier. These results justify and support the interpretation given to the results of experimental carcinogenicity studies on coal dust which never succeeded in demonstrating a higher incidence of cancer, particularly lung cancer, in treated animals.

Gill JH, James NH, Roberts RA, Dive C. **The non-genotoxic hepatocarcinogen nafenopin suppresses rodent hepatocyte apoptosis induced by TGFbeta1, DNA damage and Fas**. *Carcinogenesis* 1998;19(2):299-304.

The suppression of apoptosis may contribute to the carcinogenicity of the peroxisome proliferators (PPs), a class of non-genotoxic rodent hepatocarcinogens. Our previous work demonstrated that the PP nafenopin suppressed both spontaneous and transforming growth factor beta1 (TGFbeta1)-induced hepatocyte apoptosis both in vivo and in vitro. Here, we extend these observations by demonstrating the ability of nafenopin to suppress apoptosis induced by other major candidates for the signalling of cell death in the liver. Treatment of rat or mouse hepatocyte monolayers with TGFbeta1 or the DNA damaging drugs etoposide or hydroxyurea induced high levels of apoptosis. Western blot analysis did not support a role for either p53 or p21waf1 in etoposide-induced apoptosis in rat hepatocytes. Treatment of mouse hepatocytes with an agonistic anti-Fas antibody also resulted in an induction of high levels of apoptosis. Pre-addition and continued exposure to nafenopin suppressed apoptosis induced by all three stimuli. Overall, our studies demonstrate that the ability of nafenopin to protect hepatocytes from apoptosis is not restricted to species or apoptotic stimulus. It is possible, therefore, that the PPs may suppress apoptosis by acting on diverse signalling pathways. However, it seems more likely that nafenopin suppresses hepatocyte apoptosis elicited by each death stimulus by impinging on a core apoptotic mechanism.

Haas MJ, Pitot HC. **Characterization of rare p53 mutants from carcinogen-treated albumin-simian virus 40 T-antigen transgenic rats.** *Mol Carcinog* 1998;21(2):128-34.

The p53 gene has been either mutated or deleted in most human tumors examined to date. Mutations in the specific DNA-binding domain are the most common p53 mutations and are of interest because they may produce p53 molecules with transcriptional capabilities unlike those of the wild-type (WT) p53 protein. Mutations in the rat p53 gene were found in hepatic neoplasms of carcinogen-treated transgenic rats that express simian virus 40 (SV40) large T-antigen (TAg). Because this result was unexpected, we examined some of the biochemical and biological properties of the mutant proteins. Corresponding nucleotide changes were made by site-directed mutagenesis of the rat p53 cDNA, which was then inserted into a eukaryotic expression vector and transfected into the human hepatocyte cell line Hep 3B. Four of the mutant p53 molecules from rat hepatomas retained a strict WT conformation. Two others existed in both WT and mutant conformations. All of the mutant proteins were able to bind TAg as well as WT p53 did. Whereas the WT p53 protein was able to repress expression of a reporter gene containing a p53-response element (pSV2CAT), the missense-mutant p53 proteins induced transcription of the reporter to an extent equivalent to that of TAg. The mutant proteins also allowed TAg to induce the pSV2CAT reporter gene. The mutant molecules were able to enhance survival of Hep 3B cells, perhaps by preventing cell death, whereas expression of the WT p53 protein caused a reduction in cell number to nearly 10% of control levels. The results of these experiments suggest that the mutant p53 molecules observed in the carcinogen-treated transgenic rats may have unique properties that are important in carcinogenesis.

Hainaut P, Vahakangas K. **p53 as a sensor of carcinogenic exposures: mechanisms of p53 protein induction and lessons from p53 gene mutations.** *Pathol Biol* 1997;45(10):833-44.

BIOSIS COPYRIGHT: BIOL ABS. The p53 tumor suppressor gene encodes a nuclear phosphoprotein with growth inhibiting properties, which is activated in cell exposed to various forms of DNA damaging stress. The development of human cancer often involves inactivation of this suppressor through various mechanisms, including gene deletions and point mutations. Most mutations impair the specific DNA-binding capacity of p53, therefore allowing cells to proliferate in conditions where cells with intact p53 function are suppressed or eliminated. Thus, mutation of p53 may provide a selective advantage for the clonal expansion of preneoplastic or neoplastic cells. The diversity of p53 mutations provides a valuable tool to identify important sources of cancer-causing mutation in the human setting. Mutagens and carcinogens damage the genome in characteristic ways, leaving mutagen fingerprints in DNA. Well-characterized examples of such fingerprints include G: C to T: A transversions in lung cancers in association with cigarette smoke, G: C to T: A transversions at codon 249 in liver cancers in association with dietary exposure to Aflatoxin B1 (AFB1) and CC: GG to TT: AA tandem dipyrimidine transitions in skin cancers in association with UVB exposure. In addition, mutations at different codons are not functionally equivalent. The availability of crystal structures of p53 protein represents an essential development in the understanding of the functional properties of p53 mutants. In the future, it is expected that analysis of p53 mutations may provide useful information for the diagnosis, prognosis and therapy of cancer.

Kerckaert GA, Leboeuf RA, Isfort RJ. **Use of the Syrian hamster embryo cell transformation assay**

for determining the carcinogenic potential of heavy metal compounds. *Fundam Appl Toxicol* 1996 Nov;34(1):67-72.

Cobalt sulfate hydrate, gallium arsenide, molybdenum trioxide, vanadium pentoxide, and nickel sulfate heptahydrate were tested in the Syrian hamster embryo (SHE) assay in order to increase the SHE assay database for heavy metals. All five compounds produced significant morphological transformation at one or more doses in a dose-responsive manner. Cobalt sulfate hydrate, gallium arsenide, molybdenum trioxide, and nickel (II) sulfate heptahydrate were all positive with a 24-hr exposure, suggesting direct DNA perturbation. Vanadium pentoxide was negative with a 24-hr exposure, but positive with a 7-day exposure. This pattern of response (24-hr SHE negative/7-day SHE positive) has been seen with other chemicals which have tumor promotion-like characteristics. Since the inception of the use of the SHE cell transformation assay for detecting the neoplastic transformation potential of chemicals, over 42 heavy metal compounds have been tested in this assay. Based on the 24 metal compounds which have been tested in the SHE, Salmonella, and some type of rodent bioassay, the SHE assay is 92% concordant with rodent bioassay carcinogenicity results, including a sensitivity of 95% (21/22) and a specificity of 50% (1/2). At this time, the measure of SHE assay specificity for rodent carcinogenicity of metals is limited by the paucity of metal compounds which are rodent noncarcinogens. The Salmonella assay results are only 33% concordant with the rodent bioassay for these same chemicals. This relatively high concordance between the SHE assay and the rodent bioassay carcinogenicity results demonstrates the utility of the SHE assay for determining the carcinogenic potential of heavy metal compounds in rodent cancer bioassays.

Khan QA, Vousden KH, Dipple A. **Cellular response to DNA damage from a potent carcinogen involves stabilization of p53 without induction of p21(waf1/cip1).** *Carcinogenesis* 1997;18(12):2313-8.

The effect of a potent mammary carcinogen, anti benzo[g]chrysene 11,12-dihydrodiol 13,14-epoxide, on the progress of human mammary carcinoma MCF-7 cells through the cell cycle was investigated. While these cells, which express wild-type p53, were arrested in G1 after treatment with actinomycin D (a positive control), treatment with the mammary carcinogen did not cause G1 arrest but instead delayed the cells in the DNA synthesis phase. In concert with the absence of a G1 arrest, it was found that though both chemical treatments led to increased levels of p53, only the p53 induced by actinomycin D was transcriptionally active and increased the levels of the cyclin dependent kinase inhibitor, p21(waf1/cip1). Since treatment of the cells with the mammary carcinogen did not abrogate the G1 arrest induced by actinomycin D, the lack of p21(waf1/cip1) and of G1 arrest, resulting from treatment with the mammary carcinogen alone, was not due to some general inhibition of transcription or translation. An analogous difference between these two chemicals was demonstrated also in other human cell systems. The stealth-like property of the mammary carcinogen that allows it to damage DNA without turning on the cells' 'guardian of the genome' defense mechanism presumably increases the likelihood of malignant change because DNA replication continues on a damaged template. It is suggested that this stealth characteristic may be a major contributor to the high carcinogenic potency of this mammary carcinogen and possibly to that of other highly potent carcinogens.

Kolesnichenko TS, Gor'kova TG. **[Induction by benz(a)pyrene of squamous cell metaplasia of the respiratory epithelium in organ cultures: importance of the route of administration of the**

carcinogen and genetic susceptibility of the animals]. Arkh Patol 1996 Jul-Aug;58(4):56-61. (Rus)

Lewis DF, Ioannides C, Parke DV. **Further evaluation of COMPACT, the molecular orbital approach for the prospective safety evaluation of chemicals.** Mutat Res 1998;412(1):41-54. The molecular dimensions and electronic structures of the first group of 100 US NCI/NTP miscellaneous chemicals, evaluated for potential carcinogenicity by computer-optimized molecular parametric analysis for chemical toxicity (COMPACT) have been re-determined. Using improved criteria for cytochrome P450 (CYP) substrate specificity, re-defined for CYP1 as having a COMPACT radius [square root of $(\Delta E - 9.5)^2 + (a/d(2) - 7.8)^2$] of < 6.5 , and for CYP2E as having a collision diameter of 6.5 angstroms or less and $\Delta E < 15.5$, the likely substrates of CYP1 and CYP2E, which are regarded as potential carcinogens, have been identified. In addition, log P values have been taken into account; those chemicals with $\log P < 0$ are non-lipophilic substrates unlikely to reach the activating cytochrome enzymes, and have been regarded as non-carcinogens. The second group of 100 US NCI/NTP chemicals have also now been categorized by COMPACT into CYP1 and CYP2E substrates, and their potential carcinogenicities evaluated. Of the 203 chemicals in the 2 groups, those positive in the rodent two-species life-span carcinogenicity study (rodent assay) were 53%, those positive in the Ames test (mutagenicity) were 48%, and those positive in the COMPACT programme (carcinogenicity, mutagenicity, cytotoxicity) were 54%. Concordance between the COMPACT prediction of carcinogenicity/cytotoxicity and rodent two species life-span carcinogenicity data for the 203 chemicals is 69%, and correlation of COMPACT with Ames test data is 61%. The sensitivity of COMPACT for predicting rodent carcinogenicity is 72%, whereas the sensitivity of the Ames test for predicting carcinogenicity for the 203 chemicals was only 57%. The degree (severity) of rodent carcinogenicity also showed correlation with the COMPACT predictive evaluations of the chemicals.

Lin HL, Roberts ES, Hollenberg PF. **Heterologous expression of rat P450 2E1 in a mammalian cell line: in situ metabolism and cytotoxicity of N-nitrosodimethylamine.** Carcinogenesis 1998;19(2):321-9.

GM0637, a human fibroblast cell line, was transfected with pCMV2E1, an expression vector containing the full length cDNA for rat cytochrome P450 2E1 (P450 2E1), and with pCMVneo, which contained vector alone, and the selected clones were designated GM2E1 and GMneo, respectively. Western blot analysis showed that GM2E1, but not GMneo, expressed a protein that reacted with anti-human P450 2E1 antibody. The 7-ethoxycoumarin O-deethylase, p-nitrophenol hydroxylase, and N-nitrosodimethylamine (NDMA) demethylase activities of the P450 in these cells were measured in monolayer cell cultures without preparing microsomes. Exposure of the GM2E1 cells to NDMA for 4 days caused severe decreases in cell viability, as determined by crystal violet uptake, and showed a sigmoidal dose-response curve with a median lethal dose of 17 microM. In contrast, the viability of GMneo cells was not altered by NDMA even at concentrations up to 10 mM. Time- and concentration-dependent methylation of DNA, RNA and protein by [¹⁴C]NDMA was only observed in cells expressing P450 2E1. Inhibitors of P450 2E1 activity such as ethanol, 4-methylpyrazole, and isoniazid caused a 90% decrease in the methylation of cellular macromolecules and also completely protected the cells against NDMA-mediated toxicity. The cytotoxicity due to exposure to NDMA was partially inhibited by antioxidants such as N-acetylcysteine, ascorbic acid, butylated hydroxyanisole and N-t-butyl-alpha-phenylnitronone but was not potentiated upon glutathione depletion. These results document

the ability of rat P450 2E1 to metabolize NDMA to toxic reactive intermediates and demonstrate that this cell line provides a useful model for studying the mechanisms of metabolism-mediated toxicity and carcinogenesis.

McDougal A, Safe S. **Induction of 16alpha-/2-hydroxyestrone metabolite ratios in MCF-7 cells by pesticides, carcinogens, and antiestrogens does not predict mammary carcinogens.** Environ Health Perspect 1998;106(4):203-6.

The effects of several pesticides, mammary carcinogens, and antiestrogens on 17beta-estradiol (E2), 16alpha- and 2-hydroxylase activities, and 16alpha-/2-hydroxyestrone (OHE1) ratios were investigated in MCF-7 cells using a radiometric assay. The mammary carcinogens 7,12-dimethylbenz[a]anthracene (DMBA) and benzo[a]pyrene (BaP), respectively, increased and decreased 16alpha-/2-OHE1 ratios at some concentrations. The 16alpha-/2-OHE1 metabolite ratios for 10(-5) M kepone, atrazine, p,p'-DDE, o,p'-DDE, o,p'-DDT, and beta-hexachlorocyclohexane were 1.82 +/- 0.060, 0.71 +/- 0.027, 0.66 +/- 0.030, 1.56 +/- 0.089, 1.14 +/- 0.059, and 0.69 +/- 0.052 (mean +/- standard error), respectively, and did not show any specific trend. The effects of a series of direct and indirect acting antiestrogens on 16alpha-/2-OHE1 metabolite ratios were also investigated, and the results were compound specific. Indole-3-carbinol, tamoxifen, 4'-hydroxytamoxifen, and 9-cis-retinoic acid decreased the ratio; the effects of all trans-retinoic acid and 2,3,7,8-tetrachlorodibenzo-p-dioxin were concentration dependent; the antiestrogen ICI 182,780 increased the 16alpha-/2-OHE1 metabolite ratio. The results indicate that in MCF-7 cells treated with pesticides, mammary carcinogens, and antiestrogens, there were both increased and decreased 16alpha-/2-OHE1 metabolite ratios for each class of chemicals and the assay did not predict mammary carcinogens.

Miura M, Karasaki Y, Abe T, Higashi K, Ikemura K, Gotoh S. **Prompt activation of telomerase by chemical carcinogens in rats detected with a modified TRAP assay.** Biochem Biophys Res Commun 1998;246(1):13-9.

The maintenance of telomere length is crucial for survival of cells. Telomerase is an RNA-containing reverse transcriptase, which is responsible for elongation of shortened telomeres. Telomerase reactivation has been suggested to be involved in malignant progressions. To study on the involvement of telomerase activation in in vivo carcinogenesis, we first modified the original TRAP assay by changing the primer designs and the labeling method of PCR products to an end-labeling method. Second, we investigated the activation of telomerase in different organs after treatments of rats with various chemical carcinogens. Very early after the beginning of the treatment, telomerase activity in the liver, kidney, and lung was increased. In most cases, telomerase activation occurred in the primary or favorite target organs. The present results suggest that telomerase activation occurs promptly when animals are exposed to chemical carcinogens, which may contribute to in vivo chemical carcinogenesis.

Moll UM, Schramm LM. **p53--an acrobat in tumorigenesis.** Crit Rev Oral Biol Med 1998;9(1):23-37. The p53 tumor suppressor protein plays a central role in maintaining genomic integrity. It does so by occupying a nodal point in the DNA damage control pathway. When cells are subject to ionizing radiation or other mutagenic events, p53 mediates cell cycle arrest or programmed cell death (apoptosis). Furthermore, some evidence suggests that p53 plays a role in the recognition and repair of damaged DNA. Biochemically, p53 is a sequence-specific transcriptional stimulator and a non-specific

transcriptional repressor but also engages in multiple protein-protein interactions. Conversely, disruption of the p53 response pathway strongly correlates with tumorigenesis. p53 is functionally inactivated by structural mutations, neutralization by viral products, and non-mutational cellular mechanisms in the majority of human cancers. p53-deficient mice have a highly penetrant tumor phenotype, with over 90% tumor incidence within nine months. In some cancers, direct physical evidence exists identifying the p53 gene as a target of known environmental carcinogens such as UV light and benzolalpyrene in cancers of the skin and lung. When p53 loss occurs, cells do not get repaired or eliminated but rather proceed to replicate damaged DNA, which results in more random mutations, gene amplifications, chromosomal rearrangements, and aneuploidy. In some experimental models, loss of p53 confers resistance to anticancer therapy due to loss of apoptotic competence. The translational potential of these discoveries is beginning to be tested in novel p53-based therapies.

Nelson HH, Wiencke JK, Gunn L, Wain JC, Christiani DC, Kelsey KT. **Chromosome 3p14 alterations in lung cancer: evidence that FHIT exon deletion is a target of tobacco carcinogens and asbestos.** *Cancer Res* 1998;58(9):1804-7.

Alterations in the FHIT gene region have been previously associated with smoking status and the occurrence of lung tumors. In the current study, we examined the nature of the mutations that occur at FHIT and the types of carcinogen exposures that are associated with FHIT alterations. We screened 40 primary lung tumors for the presence of point mutations within the coding exons of FHIT using PCR-single-strand conformational polymorphism. Tumors were also analyzed for allelic loss using microsatellite markers located in or near FHIT. No tumors contained point mutations within the coding region of the FHIT gene. However, several samples failed to generate a PCR product, suggesting that regions of the gene are homozygously deleted. Samples were reanalyzed for exon loss using PCR; 13 of 30 tumors failed to generate a PCR product, and 20 of 30 tumors were missing at least one FHIT exon or had loss (loss of heterozygosity or deletion) of one microsatellite marker, suggesting that regions of the gene are homozygously deleted. These data indicate that the FHIT gene has a novel pattern of mutational inactivation not seen previously with other tumor suppressor genes, most likely influenced by the proximity of the FRA3B region. There were no associations of age, sex, p53, or k-ras mutation and FHIT exon deletion. However, there was an association of smoking duration and asbestos exposure with FHIT exon loss, indicating that carcinogenic exposures may be causal in the generation of alterations in the FHIT region.

Neumann HG, Thielmann HW, Filser JG, Gelbke HP, Greim H, Kappus H, Norporth KH, Reuter U, Vamvakas S, Wardenbach P, et al. **Proposed changes in the classification of carcinogenic chemicals in the work area.** *Regul Toxicol Pharmacol* 1997;26(3):288-95.

Noguchi M, Nomata K, Watanabe J, Kanetake H, Saito Y. **Changes in the gap junctional intercellular communication in renal tubular epithelial cells in vitro treated with renal carcinogens.** *Cancer Lett* 1998;122(1-2):77-84.

Gap junctional intercellular communications (GJIC) are known as the channels for the direct transfer of cytoplasmic molecules between neighboring cells and are lost during transformation of normal cells. To study the function and the molecular mechanism for the loss of GJIC, the effects of dimethylnitrosamine, KBrO₃ and FeSO₄ x 7H₂O, which are known as chemical tumor promoters of the

kidney on the GJIC function and the expression of connexin 43 of Madin-Darby canine kidney (MDCK) epithelial cells, were examined. These tumor promoters inhibited the GJIC in MDCK cells. The expression of connexin 43 mRNA and connexin 43 protein was not altered by these treatments, whereas immunocytochemical study revealed that the distribution of connexin 43 protein was changed from the cell surface to the cytoplasm. These data suggest that blockage of GJIC in MDCK cells treated with renal carcinogens support the hypothesis that loss of GJIC might be important in renal carcinogenesis.

Portier C, El Masri H. **Statistical research needs in mechanistic modelling for carcinogenic risk assessment.** Stat Methods Med Res 1997;6(4):305-15.

If the broad spectrum of mechanistic research conducted on an environmental carcinogen is to be used in quantifying cancer risks, statisticians must play a key role. Statistical methods are critically needed for a scientifically valid analysis of a complicated series of linked experimental findings. This will require a greater understanding of the underlying biology than is common in statistical consulting, aiding in the development of complicated mechanistically based mathematical descriptions of mean response and in the creation of statistical methods for the estimation of model parameters (e.g. likelihoods) able to use both the underlying model and much of the available data.

Primiano T, Sutter TR, Kensler TW. **Redox regulation of genes that protect against carcinogens.** Comp Biochem Physiol B Biochem Mol Biol 1997;118(3):487-97.

Most carcinogens require activation to electrophilic metabolites or species that generate reactive oxygen in order to initiate the tumorigenic process. These reactive intermediates can, in turn, be detoxified by endogenous enzyme systems that and in the protection of cells from either toxic or mutagenic product formation. The levels of many of these enzymes are elevated by numerous compounds found in the diet, or by antioxidants. Recent evidence describes the mechanism for this induction of carcinogen detoxication enzymes to be regulated at the transcriptional level. Nuclear transcription factors bound to sites common among these carcinogen detoxication genes are activated by as yet unknown signal transduction pathways. The activity of these nuclear transcription factors are modulated by pro- and antioxidant reagents, suggesting that a redox-sensitive component governs the induction of enzymes involved in carcinogen metabolism. In this review, evidence for the redox regulation of the genes encoding carcinogen detoxication enzymes is presented. Evidence is also presented suggesting the participation of nuclear factor kappa B (NF-kappa B), mitogen-activated protein (MAP) kinase, and basic leucine zipper (bZIP) proteins and their activation pathways in this induction.

Rosenkranz M, Rosenkranz HS, Klopman G. **Intercellular communication, tumor promotion and non-genotoxic carcinogenesis: relationships based upon structural considerations.** Mutat Res 1997;381(2):171-88.

An SAR model for inhibition of metabolic cooperation (iMC) was developed. The structural and physicochemical features associated with the ability to cause iMC are primarily lipophilic moieties consistent with the possibility that they represent receptor-binding ligands. There are also significant parallels between the structural descriptors associated with iMC and those associated with tumor promotion and with carcinogenesis in rodents. Overall, the present study provides structural evidence that iMC is a feature associated with the carcinogenic process.

Schuller HM, Orloff M. **Tobacco-specific carcinogenic nitrosamines: Ligands for nicotinic acetylcholine receptors in human lung cancer cells.** *Biochem Pharmacol* 1998;5(9):1377-84. BIOSIS COPYRIGHT: BIOL ABS. Lung cancer demonstrates a strong etiologic association with smoking. Of the two most common histologic lung cancer types, small cell carcinoma (SCLC) is found almost exclusively in smokers, whereas peripheral adenocarcinoma (PAC) also develops in a significant number of nonsmokers. N'-Nitrosonicotine (NNN) and 4(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), nicotine-derived nitrosamines, are potent lung carcinogens contained in tobacco products. Because of the structural similarity of NNN and NNK with nicotine, we hypothesized that these carcinogens are ligands for nicotinic acetylcholine receptors (nAChRs). Using cell lines derived from human small cell lung carcinoma and pulmonary adenocarcinoma with the site-selective ligands alpha-bungarotoxin (alpha-BTX) and epibatidine (EB) in receptor binding and cell proliferation assays, we found that SCLC expressed neuronal nicotinic receptors with high affinity to alpha-BTX, whereas PAC cells expressed nicotinic receptors with high affinity to EB. NNK bound with high affinity to alpha-BTX-sensitive nAChRs in SCLC cells, while NNN bound with high affinity to EB-sensitive nAChRs in PAC cells. The affinity of each nitrosamine to these receptors was several orders of magnitude greater than that of nicotine. NNK stimulated the proliferation of SCLC cells via this mechanism. Our findings suggest that NNK may contribute to the genesis of SCLC in smokers via chronic stimulation of the alpha-BTX-sensitive nAChR-subtype expressed in these cells. Both nitrosamines may also contribute to a host of nicotine-related diseases that are currently thought to be caused by the chronic interaction of nicotine with nAChRs expressed in a large spectrum of mammalian cells.

Trump BF. **The Twelfth Aspen Cancer Conference: Meeting on the Mechanisms of Toxicity and Carcinogenesis, Aspen, Colorado, USA, July 11-16, 1997.** *Toxicol Pathol* 1997;25(6):674-96. BIOSIS COPYRIGHT: BIOL ABS. This meeting contains abstracts of 28 papers, written in English, covering DNA repair, cell death, translational research, status of molecular diagnosis, carcinogen assessment strategy, mechanistic paradigms in risk assessment, cancer therapy, and tumor progression.

Tsao H, Mao B, Zhuang P, Xu R, Amin S, Geacintov NE. **Sequence dependence and characteristics of bends induced by site-specific polynuclear aromatic carcinogen-deoxyguanosine lesions in oligonucleotides.** *Biochemistry* 1998;37(14):4993-5000. BIOSIS COPYRIGHT: BIOL ABS. The tumorigenic metabolite of benzo(a)pyrene, the (+)-7R,8S,9S,10R enantiomer, and the nontumorigenic mirror-image isomer, (-)-7S,8R,9R,10S, of r7,t8-dihydroxy-t9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (anti-BPDE) bind covalently to the exocyclic amino group of deoxyguanosine (N2-dG) in native DNA. These adducts can cause structural perturbations such as DNA bends, which in turn may influence the cellular processing of these lesions. The characteristics of bends in site-specifically modified oligodeoxyribonucleotide duplexes induced by single (+)- and (-)-anti-(BP)-N2-dG lesions were examined by self-ligation and gel electrophoresis techniques. The modified residues (dG*) were centrally positioned in the 11-mer oligonucleotide d(CACAXG*XACAC) complexed with the natural complementary strands, with X = T or C, or in oligonucleotides 16 or 22 base pairs long with the same centrally positioned 11-mer. Among the four stereochemically distinct lesions, the 10S (+)-trans-anti-(BP)-N2-dG adducts were significantly more bent than any of the other three stereoisomeric adducts and were selected for detailed studies. In the TG*T sequence context (X = T), the retardation factor RL (apparent length of multimer/sequence

length) is approximately independent of the phasing (distance, in base pairs, between the lesions) of the adducts with respect to the helical repeat (10.5 base pairs/helix turn). In contrast, in the CG*C sequence context (X = C), RL is markedly lower in the case of ligated 16-mers than in the case of ligated 11-mer duplexes. The dependence of RL on the phasing of the bends as a function of the helical repeat, indicate that the bends associated with (+)-trans-anti-(BP)-N2-dG lesions are relatively rigid in the d(...CG*C...) d (...GCG...) sequences, and flexible in the d(...TG*T...) d(...ACA...) sequence context. These differences are attributed to the orientations of the pyrenyl residues on the 5'-side of the modified deoxyguanosine residues in the minor groove and to the intrinsic roll and tilt characteristics of DNA dinucleotide steps CG, GC, TG, and GT. The influence of flanking bases on the extent and character of DNA bending suggest that base sequence effects may be important in the cellular processing of (+)-trans-anti-(BP)-N2-dG lesions.

Woo YT, Lai D, Arcos J, Argus M, Cimino M, Devito S, Keifer L. **Mechanism-based structure-activity relationship SAR analysis of carcinogenic potential of 30 NTP test chemicals.** J Environ Sci Health C 1997;15(2):139-60.

BIOSIS COPYRIGHT: BIOL ABS. RRM LITERATURE REVIEW STRUCTURE-ACTIVITY RELATIONSHIPS NTP TEST CHEMICALS CARCINOGENIC POTENTIAL TOXICOLOGY TOXIC SUBSTANCES CONTROL ACT EPA HAZARD ASSESSMENT CARCINOGENESIS GOVERNMENT AGENCY.

Zhou L, Cho BP. **Synthesis, characterization, and comparative conformational analysis of N-(deoxyguanosin-8-yl)aminopyrene adducts derived from the isomeric carcinogens 1-, 2-, and 4-nitropyrene.** Chem Res Toxicol 1998;11(1):35-43.

Nitrated polycyclic aromatic hydrocarbons are mutagens/carcinogens that undergo in vivo activation by ring-oxidation and nitro-reduction pathways. We report the syntheses and comparative conformational analyses of N-(deoxyguanosin-8-yl)-n-aminopyrene adducts (dG-C8-n-AP, n = 1, 2, 4) derived from the three isomeric mononitropyrenes (1-, 2-, and 4-NP). The C8-amine nitrogens of these adducts have been enriched with ¹⁵N to examine the conformation about the pyrenyl-nitrogen and guanyl-nitrogen bonds that link the guanine and the pyrene moiety. These adducts are structurally isomeric, thus providing an interesting opportunity for systematic probing of the isomeric adduct conformations. Spectroscopic data indicated that the three isomeric aminopyrene adducts favor anti-glycosyl conformations, with C2'-endo (S) sugar puckering and a nearly planar conformation at the central amine nitrogen. The data further indicated differences in the extent of pi-electron conjugations about the pyrenyl-nitrogen bond, depending on the location of aminopyrene substitution. Thus while the 1- and 4-isomers both have substitution adjacent to a fused aromatic ring, the 2-isomer is highly symmetric and less sterically hindered. The 2-isomer adopts the most planar conformation, thereby having the most efficient pi-electron delocalization between the guanine and pyrene rings. The isomeric dG-C8-AP adducts and their nitro and amino precursors display physicochemical properties (HPLC retention time, UV pattern, ¹H NMR data, mass fragmentation, etc.) distinctly dependent on their structures (1- and 4-isomers versus 2-isomer).

CYTOTOXICITY

Burney S, Tamir S, Gal A, Tannenbaum SR. **A mechanistic analysis of nitric oxide-induced cellular toxicity.** Nitric Oxide 1997;1(2):130-44.

BIOSIS COPYRIGHT: BIOL ABS. Nitric oxide (NO)-induced toxicity was investigated in two different cell lines, Chinese hamster ovary (CHO-AA8) and human lymphoblastoid (TK6), over a range of NO doses (0-9 mM) delivered for an exposure of 2 h. To determine both short-term and delayed effects leading to death, a range of assays was employed to decipher the major mechanisms of cytotoxicity. Examples of damage parameters measured in this study include inhibition of DNA synthesis, damage to mitochondria, loss of cell membrane integrity, apoptosis, changes in cell cycle distribution, and the occurrence of DNA strand breaks. Our results indicate that NO-induced toxicity is an extremely complex process involving multiple pathways generally leading to apoptotic cell death. Results consistently demonstrate that TK6 cells are much more susceptible to NO-induced toxicity than CHO-AA8 cells. This difference in sensitivity could be seen for all types of cellular damage examined. The earliest observable effect of NO exposure is inhibition of DNA synthesis which is not the result of inhibition of ribonucleotide reductase but may be the result of DNA damage leading ultimately to cell cycle arrest.

Chang YC, Tai KW, Cheng MH, Chou L S, Chou MY. **Cytotoxic and non-genotoxic effects of arecoline on human buccal fibroblasts in vitro.** J Oral Pathol Med 1998;27(2):68-71.

BIOSIS COPYRIGHT: BIOL ABS. Betel quid chewing has been linked to oral submucous fibrosis and oral cancer. Cytotoxicity and genotoxicity assays were used to investigate the pathobiological effects of arecoline on cultured human buccal fibroblasts. Arecoline increased double-stranded polynucleic acid at the concentration of 0.1 to 10 mug/ml in a concentration-dependent manner. At a concentration higher than 50 mug/ml, arecoline was cytotoxic to cultured fibroblasts and the cytotoxicity was dose-dependent. No genotoxicity for arecoline was found even at a concentration of 400 mug/ml. On the other hand, 600 mug/ml glutathione (GSH) and 200 mug/ml glycyrrhizin could prevent the arecoline-induced cytotoxicity. These results indicate that arecoline is a cytotoxic agent and no genotoxicity was found to human buccal fibroblasts. Furthermore, increasing consumption of GSH- and glycyrrhizin-rich foods may reduce the oral diseases associated with betel quid chewing.

Ciapetti G, Granchi D, Verri E, Savarino L, Stea S, Savioli F, Gori A, Pizzoferrato A. **False positive results in cytotoxicity testing due to unexpectedly volatile compounds.** J Biomed Mater Res 1998;39(2):286-91.

We investigated the cytotoxicity of different dental materials according to the study protocol adopted by our lab for the screening of new materials. Experimental parameters used in such testing are addressed mainly in documents EN 30993 Biological evaluation of medical devices, Part 5: Tests for cytotoxicity: in vitro methods and Biological evaluation of medical devices, Part 12: Sample preparation and reference materials. Cells were cultured in microplates and challenged with aqueous extracts of the materials. The assay methods were neutral red- and propidium iodide-uptake assays, both indicative of cell viability and able to provide quantitative data. The observation of contrasting results for one material using the above-mentioned methods raised some concern about the assay system used. With further experimentation, it appeared that a sustained release of volatile substances still present in one extract exerted a toxic effect in neighboring cultures. It is concluded that in the microenvironment of a microplate the distribution of samples cannot be disregarded, as it may be responsible for toxicity cross-

contamination. Moreover, the use of more than one single method has to be recommended in cytotoxicity testing, in order to avoid false positive results due to experimental artifacts.

Dikomey E, Dahm-Daphi J, Brammer I, Martensen R, Kaina B. **Correlation between cellular radiosensitivity and non-repaired double-strand breaks studied in nine mammalian cell lines.** *Int J Radiat Biol* 1998;73(3):269-78.

PURPOSE: To test the relationship between cell killing and non-repaired DNA strand breaks both in repair proficient and deficient cell lines. **MATERIALS AND METHODS:** Five of the cell lines used are repair competent (CHO, CHO K1, rat rhabdomyosarcoma R1H, mouse balb and normal human fibroblasts), while four display a reduced repair capacity (scid, xrs1, xrs5, AT). Cell survival was determined by colony formation assay. The total number of strand breaks was measured by the alkaline unwinding technique and the numbers of double-strand breaks by constant-field gel electrophoresis. **RESULTS:** The nine cell lines showed a broad spectrum in radiosensitivity with SF2 values ranging from 0.018 to 0.58. The cell lines did not vary in the number of induced strand breaks, neither for all strand breaks nor for double-strand breaks alone. In contrast, there was a large variation in the number of non-repaired strand breaks measured 24 h after irradiation. Comparison of cell killing with the number of non-repaired breaks measured after a dose of 90 Gy showed no correlation for single-strand breaks ($r^2=0.29$) but a fairly good correlation for double-strand breaks ($r^2=0.87$). This correlation was found to hold both for repair proficient and deficient cell lines. **CONCLUSIONS:** The results obtained strongly suggest that the number of non-repaired double-strand breaks measured 24h after irradiation can be used as an indicator of cellular radiosensitivity.

Guandalini E, Ioppolo A, Mantovani A, Stacchini P, Giovannini C. **4-Hexylresorcinol as inhibitor of shrimp melanosis: efficacy and residues studies; evaluation of possible toxic effect in a human intestinal in vitro model (Caco-2); preliminary safety assessment.** *Food Addit Contam* 1998;15(2):171-80.

Studies were performed on the efficacy, residues and in vitro enterocyte toxicity of 4-hexylresorcinol (4-HR), which could be utilized as an inhibitor of shrimp melanosis (black spot). Mediterranean sea shrimp (*Parapaeneus longirostris*) were treated with solutions of 4-HR in sea-water, at three different concentrations, 25, 50 or 100 mg/kg of shrimp, to test its antioxidative property. As a comparison a group of shrimp was treated with sodium metabisulphite (1 g/kg), while another group was left untreated. 4-HR showed a marked ability to inhibit or slow down melanosis (black spot) in shrimp; the most effective concentration was 100 mg/kg within an optimum period of 7 days but with effects up to the tenth day. During the first 5 days, 4-HR residues in the edible part of the shrimp showed a fast decrease in all three groups, going from initial average values of 20 mg/kg at 0 time, to 0.9 in the group treated at 25 mg/kg; from 42 to 1.8 mg/kg in the group at 50 mg/kg and from 85 to 1.9 mg/kg in the group at 100 mg/kg. In vitro studies on enterocyte-like Caco-2 cells did not indicate any cytotoxic effect up to a concentration of 50 micrograms/ml. Moreover, no inhibition of protein synthesis was observed, which lends further support to the absence of significant damage to the intestinal mucosa induced by 4-HR. The available database on 4-HR pharmacology and toxicology is inadequate to determine even a provisional ADI. There is negative evidence of carcinogenesis and no significant untoward effects were observed in humans when it was used as an anthelmintic. However, it is not possible to determine a NOEL for non-genotoxic effects. 4-HR could become an interesting alternative to the use of sulphites to

prevent black spot. However, a more complete database is needed to achieve a regulatory evaluation.

Kerr DE, Wu GY, Wu CH, Senter PD. **Listeriolysin O potentiates immunotoxin and bleomycin cytotoxicity.** *Bioconjug Chem* 1997;8(6):781-4.

Antitumor immunotoxins were formed by covalently attaching the ribosome-inactivating protein ricin A chain (RA) to the antitumor antibodies BR96 and L6. In vitro cytotoxicity assays established that BR96-RA was cytotoxic to H2987 human lung adenocarcinoma cells (IC₅₀ = 6 nM), while L6-RA exhibited very low levels of cytotoxic activity (18% cell kill at 67 nM). The virulence factor from the intracellular pathogen *Listeria monocytogenes*, listeriolysin O (LLO), was able to potentiate the cytotoxicity of BR96-RA and L6-RA by 120- and > 1340-fold, respectively, resulting in IC₅₀ values of approximately 50 pM. LLO also potentiated the cytotoxicity of the peptide anticancer drug bleomycin by a factor of > 2500 but had no effect on the cytotoxic activities of the anticancer drugs cytarabine and etoposide phosphate. In addition, LLO did not potentiate the cytotoxic activity of unconjugated ricin A chain or L6-RA on H2987 cells that were saturated with L6 prior to conjugate treatment. These results are attributed to LLO-induced alteration of the intracellular trafficking of molecules that are incorporated into acidic vesicles.

Koutsogeorgopoulou L, Maravelias C, Methenitou G, Koutselinis A. **Immunological aspects of the common food colorants, amaranth and tartrazine.** *Vet Hum Toxicol* 1998;40(1):1-4.

BIOSIS COPYRIGHT: BIOL ABS. We describe a sensitive and reproducible microassay model using human peripheral blood lymphocytes (PBL) for discrimination between the cytotoxic and immunosuppressive effects of food colorants such as amaranth and tartrazine. The cytotoxic effects of a wide range of concentrations of these substances were studied on human PBL by the colorimetric in vitro cytotoxicity assays, neutral red uptake (NR) and thiazolyl blue tetrazolium bromide (MTT). The immunotoxic properties of these 2 substances were determined by a (3H)-thymidine DNA incorporation assay on phytohemagglutinin stimulated or non-stimulated lymphocytes, as well as by a Cr51 release Natural Killer assays. The results showed clear immunosuppressive effects from the 2 substances tested, although the concentrations chosen for this study proved to be non-cytotoxic by NR and MTT cytotoxic endpoints.

Lee YA, Chung YK, Sohn YS. **Synthesis and antitumor activity of (diamine)platinum(II) complexes of benzylmalonate derivatives.** *J Inorg Biochem* 1997;68(4):289-94.

(Diamine)platinum(II) complexes of benzylmalonate derivatives as a leaving group designed in a wide range of lipophilicity and water-solubility were prepared and their antitumor activities were attempted to correlate to their lipophilicity or solubility. A good relationship was observed between their in vitro toxicity and solubility of the title complexes with the same carrier ligand, DACH (trans-(+/-)-1,2-diaminocyclohexane): The most soluble complexes are most cytotoxic whereas the least soluble complexes are least cytotoxic. However, no relationship could be established between their in vivo activity and their lipophilicity or solubility presumably due to other pharmacokinetic factors involved in vivo. The molecular structure of (IPA)2Pt(DBM).2CH3OH (IPA = isopropylamine; DBM = dibenzylmalonate) was determined by X-ray diffraction: space group P2(1)/n, a = 11.433 (3), b = 14.461 (4), c = 17.478 (4) Å, beta = 97.25 (3) degrees, z = 4, R = 0.0437.

Malich G, Markovic B, Winder C. **The sensitivity and specificity of the MTS tetrazolium assay for**

detecting the in vitro cytotoxicity of 20 chemicals using human cell lines. Toxicology 1997;124 (3):179-92.

A number of studies reported that the MTS in vitro cytotoxicity assay is a convenient method for assessing cell viability. The main features found with this assay are its ease of use, accuracy and rapid indication of toxicity. It might well be a useful tool in human health risk assessment if it can be shown that this assay also has an acceptable sensitivity and specificity. This is of interest particularly when exposure to unknown chemical substances requires the rapid detection and evaluation of toxic effects. In this study, the cytotoxicity of 20 chemicals selected from the MEIC priority list was determined with the MTS assay. Since it could be shown that interactions between detection reagents and test chemicals might influence the results of this assay, preliminary experiments were carried out such that artifactual results due to test chemical interference could be excluded from this study. IC₅₀ (50% inhibitory concentration) were established for each test chemical in two human cell lines (F1-73 and HeLa) and later compared with published toxicity data of the same chemicals established with in vitro and in vivo toxicological test systems. Direct comparisons of the data showed a generally lower sensitivity of the MTS assay, which is influenced by biological test organisms, cell type and exposure time. In terms of the specificity of the MTS assay, the results showed a good correlation between data obtained with the MTS assay and published data. The lowest correlation was found when the MTS assay was compared with in vivo studies, however, this finding corresponds well with other published in vitro-in vivo correlations. The highest correlation was found when the MTS assay was compared with test systems using human cell lines or exposure times of 3-24 h. Since the sensitivity of the MTS assay might be increased using different cell types or by extended incubation, this assay is found to provide ideal features of a good measurement system that might also be used for on site toxicological assessments.

Morgan DM. **Tetrazolium (MTT) assay for cellular viability and activity.** Methods Mol Biol 1998;79:179-83.

Poe BS, O'Neill KL. **Caffeine modulates heat shock induced apoptosis in the human promyelocytic leukemia cell line HL-60.** Cancer Lett 1997;121(1):1-6.

BIOSIS COPYRIGHT: BIOL ABS. The ability of caffeine to modulate hyperthermia induced apoptosis was investigated in the human promyelocytic cell line HL60. Mild hyperthermia has been shown to be a strong inducer of apoptosis in many cell lines of lymphoblastoid lineage. In this investigation HL60 cells were simultaneously exposed to caffeine (concentrations ranging from 0 to 20 mM), and a brief hyperthermic treatment (43 C) for 1 h and then allowed a recovery time of 12 h. Approximately 50% of a cell population receiving the hyperthermia treatment died by apoptosis within 12 h, as determined by the comet assay, whereas cells that received concomitant treatments of caffeine with heat shock displayed an apparent suppression of apoptotic induction and enhanced cell survival.

DERMAL TOXICITY

Ballantyne B, Myers RC, Blaszcak DL. **Influence of alkalization of glutaraldehyde biocidal solutions on acute toxicity, primary irritancy, and skin sensitization.** Vet Hum Toxicol 1997;39 (6):340-6.

BIOSIS COPYRIGHT: BIOL ABS. Aqueous glutaraldehyde (GA) is used at a concentration around 2%

for the cold sterilization of endoscopy and dental instruments. Stock GA solution (pH 3.1-4.5) is alkalized (pH 7.8-8.0) before use to optimize biocidal activity. The possible differential handling hazards between acidic unbuffered GA (UGA) and alkaline buffered GA (BGA) were compared for acute toxicity, primary irritancy and skin sensitizing potential using a 2.2% GA solution. Peroral LD50 values (with 95% confidence limits) in rats (combined sexes) were 3.45 (3.13-3.80) g/kg for UGA and 4.16 (3.13-5.52) g/kg for BGA; signs and gross pathology were similar. A 24-h occluded cutaneous application of 16.0 g/kg in the rabbit did not produce mortality; moderate skin irritancy was observed. No systemic effects occurred with UGA and only a few with BGA (unsteady gait, sluggishness, rapid breathing). Local skin irritation from a 4-h occluded contact with 0.5 ml was relatively minor and slightly more marked with BGA than UGA. Rats exposed to a statically generated saturated vapor atmosphere for 6 h did not show any signs or gross pathology, and only slight weight loss occurred (UGA females). Rabbit eye irritation studies (0.1 ml) showed slightly more marked conjunctival reactions with BGA, but corneal injury was marked and persistent with BGA and only slight and transient with UGA. With 0.01 ml, no corneal injury occurred, but conjunctival reaction was more marked with UGA. A guinea pig maximization study showed UGA to produce a higher sensitizing index (68% at challenge, 32% at rechallenge) than BGA (30% at challenge, 5% at rechallenge). Severity indices at challenge was also higher for UGA (0.84 (24 h), 0.47 (48 h)) than BGA (0.45 (24 h), 0.18 (48 h)). Both UGA and BGA have generally similar acute toxicity and skin irritancy; BGA has greater corneal injuring potential, and UGA has a greater skin sensitizing potential.

Bando H, Mohri S, Yamashita F, Takakura Y, Hashida M. **Effects of skin metabolism on percutaneous penetration of lipophilic drugs.** J Pharm Sci 1997 Jun;86:759-61.

IPA COPYRIGHT: ASHP The effects of skin metabolism on percutaneous penetration of drugs with high lipophilicity were studied in vitro using rat skin pretreated with and without an esterase inhibitor, isofluorophate (diisopropylphosphofluoridate; DFP). Without DFP, about 96% of the total penetrated amount appeared as metabolized p-hydroxybenzoic acid in the receptor fluid after application of butylparaben, whereas about 30% penetrated as intact form after application of propylparaben. On the other hand, metabolized p-hydroxybenzoic acid was not detected in the receptor fluid under pretreatment with DFP. DFP significantly decreased the total amount that penetrated after application of butylparaben, but it did not significantly affect that of propylparaben. It was concluded that skin metabolism directly affected the total amount that penetrated in the case of highly lipophilic drugs, and that the higher the metabolic rate to hydrophilic drugs, the greater the amount that penetrated the skin.

Baynes RE, Monteiro-Riviere NA, Qiao GL, Riviere JE. **Cutaneous toxicity of the benzidine dye direct red 28 applied as mechanistically-defined chemical mixtures (MDCM) in perfused porcine skin.** Toxicol Lett 1997;93(2-3):159-69.

Complex chemical mixtures at hazardous waste sites can potentially consist of a marker chemical and several other chemicals, each of which can have different modulating actions on the dermatotoxicity of the marker chemical and/or other components in the mixture. A total of 16 mixtures, consisting of a marker chemical direct red 28 (DR28), a solvent (80% acetone or DMSO in water), a surfactant (0 or 10% sodium lauryl sulfate, SLS), a vasodilator (0 or 180 microg methyl nicotinate, MN) and a reducing agent (0 or 2% stannous chloride, SnCl₂) were selected. Isolated perfused porcine skin flaps (IPPSFs), which have been proven to be an in vitro model for assessing absorption and toxicity, were utilized.

These mixtures did not cause severe dermatotoxicity. However, light microscopic observations depicted minor alterations (intracellular and intercellular epidermal edema) with DMSO mixtures than with acetone mixtures. The presence of SLS caused an alteration in the stratum corneum. Enzyme histochemical staining for alkaline phosphatase (ALP) and nonspecific esterase (NSE) revealed no significant treatment effects, but increased staining for acid phosphatase (ACP) in the stratum basale was significant when associated with SLS or SLS + MN in DMSO mixtures. At 8 h post-dose, only DMSO mixtures containing SL + MN, SL + SnCl₂, or SLS + MN + SnCl₂ significantly increased transepidermal water loss. In conclusion, this study demonstrated that various mixtures, especially those containing SLS alter the epidermal barrier differently with complex interactions occurring simultaneously.

Baynes RE, Riviere JE. **Influence of inert ingredients in pesticide formulations on dermal absorption of carbaryl.** Am J Vet Res 1998;59(2):168-75.

OBJECTIVES: To assess the influence of solvent plus various mixtures on percutaneous absorption and disposition of the carbamate insecticide, carbaryl (CA). **ANIMALS:** Skin was obtained from the dorsum of 14 female weanling specific-pathogen-free Yorkshire pigs. **PROCEDURE:** In this 8-hour in vitro flow-through diffusion study, porcine skin sections were dosed with 40 micrograms of CA/cm² of surface area, different amounts of solvents (40 or 80% acetone or dimethyl sulfoxide [DMSO]), different amounts of a surfactant (0, 1, or 5% sodium lauryl sulfate [SLS]), an insect repellent (0 or 15% diethyl-m-toluamide [DEET]), an insecticide synergist (0 or 2% piperonyl butoxide [PB]), and a CA metabolite (40 micrograms/cm² 1-naphthol [1-NA]). **RESULTS:** In general, CA absorption was greater from acetone than from DMSO mixtures, and CA penetration into skin and stratum corneum was greater from DMSO at 8 hours. This is consistent with the flux-time profiles, which depicted initial peak flux within 2 to 3 hours for most acetone mixtures, but a slow increase in flux for DMSO mixtures. Irrespective of the solvent, increasing water content in pesticide dosing mixtures significantly increased CA absorption from SLS mixtures only. The SLS also enhanced CA absorption, especially at low solvent concentrations. The DEET significantly reduced CA absorption from acetone, but not from DMSO mixtures, and 1-NA enhanced CA absorption from acetone, but not from DMSO mixtures. Piperonyl butoxide significantly enhanced CA absorption from acetone and DMSO mixtures. However, addition of PB or PB plus SLS did not significantly increase CA flux above that observed from solvent plus surfactant mixtures. **CONCLUSIONS:** Inert ingredients can modulate percutaneous absorption of toxicologically important pesticides and their effect or activity on CA disposition is dependent on solvent specificity and solvent concentration. Whereas SLS, PB, and 1-NA can enhance pesticide absorption, DEET can reduce absorption.

Bhatia KS, Singh J. **Effect of dimethylacetamide and 2-pyrrolidone on the iontophoretic permeability of LHRH through porcine skin.** Drug Dev Ind Pharm 1997;23(12):1215-8.

IPA COPYRIGHT: ASHP The effects of penetration enhancers (PE) dimethylacetamide and 2-pyrrolidone and iontophoresis on the in vitro permeability of gonadorelin (luteinizing hormone-releasing hormone; LHRH) through porcine epidermis are reported. The permeability coefficient of gonadorelin increased through PE-treated epidermis. Iontophoresis further increased permeability through PE-pretreated epidermis. This demonstrated that iontophoresis can synergize with PEs to provide an additional driving force to maintain and control the target flux of gonadorelin.

Bhatia KS, Singh J. **Percutaneous absorption of LHRH through porcine skin: effect of N-methyl 2-pyrrolidone and isopropyl myristate.** Drug Dev Ind Pharm 1997;23(11):1111-4.

IPA COPYRIGHT: ASHP The effect of a penetration enhancer, N-methylpyrrolidone (N-methyl-2-pyrrolidone) or isopropyl myristate, on the in vitro permeability of gonadorelin (luteinizing hormone-releasing hormone; LHRH) through porcine epidermis was investigated. The permeability coefficient of gonadorelin significantly increased through penetration enhancer treated epidermis in comparison to the control. It was concluded that both penetration enhancers can enhance the percutaneous absorption of peptides such as gonadorelin.

Brand RM, Duensing G, Hamel FG. **Iontophoretic delivery of an insulin mimetic peroxovanadium compound.** Int J Pharm 1997 Jan 12;146:115-22.

IPA COPYRIGHT: ASHP The transdermal delivery of an insulin mimetic peroxovanadium compound across dorsal hairless mouse skin and human breast and abdominal skin was studied using iontophoresis in a flow-through diffusion cell system under different donor conditions. The compound was successfully delivered cathodally. Flux was linearly related to donor concentration and current density. The use of calcium chloride instead of sodium chloride as the donor salt significantly increased drug penetration. Transport increased with decreasing buffer concentration. Hairless mouse skin was a reasonable model for flux across human skin of this drug, since penetration across both membranes was within a factor of 2.

Forster T, Jackwerth B, Pittermann W, Von Rybinski W, Schmitt M. **Properties of emulsions.** Cosmet Toiletries 1997 Dec;112:73-6, 78-9, 81-2.

IPA COPYRIGHT: ASHP The effects of emulsion type and structure on the skin penetration of vitamins as cosmetic active ingredients were studied in vitro using the isolated perfused bovine udder skin model. The oil soluble vitamin E penetrated more strongly from water/oil than from oil/water emulsions. This was mainly attributed to the lamellar gel network blended into the oil/water emulsions to impart better consistency; this network impeded the free diffusion of the vitamin from the oil phase into the skin. The water soluble panthenol penetrated more strongly than did oil soluble vitamins; this was explained by increased thermodynamic activity when the emulsion dries on the skin.

Fuchs J, Groth N, Herrling T. **Cutaneous tolerance to nitroxide free radicals in human skin.** Free Radic Biol Med 1998;24(4):643-8.

No data are available on the irritant effect of nitroxide free radicals in human skin. Nitroxides are important biomedical skin probes used in Electron Paramagnetic Resonance spectroscopy and imaging. Our purpose was to study the skin irritation potential of different nitroxide free radical structures in skin of healthy human subjects. We investigated the following nitroxides: Tempo (2,2,6,6-tetramethyl-1-piperidinoxy), Doxo (2,2,5,5-tetramethyl-3-oxazolidinoxy), Proxo (2,2,5,5-tetramethyl- -dihydro-pyrrolinoxy), and Imidazo (2,2,3,4,5,5-hexamethyl-imidazoline-1-yloxy). Cutaneous irritation was determined in human skin following a single application and after repetitive applications in comparison to the standardized irritant sodium lauryl sulfate (SLS). The response was evaluated clinically as well as by a bioengineering method analyzing transepidermal water loss (TEWL) and skin hydration (capacitance). The nitroxides were classified clinically from nonirritant (Imidazo, Proxo), to slightly

irritant (Doxo, 100 mM), or moderately irritant (Tempo 100 mM) after a single application. The TEWL values were significantly increased by Doxo and Tempo, but capacitance values were not changed significantly. In the cumulative irritation test Tempo was scored as a slight irritant (10 mM). TOLH (2,2,6,6-tetramethyl-1-hydroxypiperidin), the hydroxylamine of Tempo, which is the major skin metabolite, did not cause skin irritation after a single or repetitive applications. This may indicate that a loss of cellular reducing equivalents may be involved in the inflammation process caused by Tempo. The order of nitroxide irritation potency (Tempo > Doxo >> Imidazo = Proxo) is inverse to the order of nitroxide biostability in human skin (Imidazo = Proxo >> Doxo > Tempo). In conclusion, nitroxide free radicals are classified as nonirritant to moderately irritant in human skin. Particularly, the pyrrolidine and imidazoline type nitroxides have a low potential to cause acute or subacute skin toxicity.

Garner CE, Matthews HB. **The effect of chlorine substitution on the dermal absorption of polychlorinated biphenyls.** Toxicol Appl Pharmacol 1998;149(2):150-8.

BIOSIS COPYRIGHT: BIOL ABS. The fate of selected mono-, di-, tetra-, and hexachlorobiphenyls was investigated following single dermal administration (0.4 mg/kg) to determine the effects of chlorine substitution on the dermal absorption and disposition of polychlorinated biphenyls (PCBs). Single dermal doses of ¹⁴C-labeled mono-, di-, tetra-, and hexachlorobiphenyls were administered to 1-cm² areas on the backs of F-344 male rats. Unabsorbed radioactivity was removed from the dose site either at euthanasia or 48 h postdose. Distribution of radioactivity in the dose site and selected tissues was determined by serial sacrifice at time points up to 2 weeks. Dermal penetration varied inversely with degree of chlorination and at 48 h ranged from ca. 100% for monochlorobiphenyl to ca. 30% for the hexachlorobiphenyl. Penetration rate constants correlated well with log K_{ow}. PCBs were retained in the epidermis for up to 2 weeks postdose. The data from these studies suggest that systemic absorption of PCBs involves a combination of sequential processes including penetration across the stratum corneum, possibly metabolism in the epidermis and/or dermis, adsorption to proteins, and finally absorption into the systemic circulation. The skin favors the rapid absorption of less chlorinated PCBs, but the relatively rapid metabolism and elimination of these compounds would result in lower body burdens. More highly chlorinated PCBs penetrate less rapidly but remain in the site of exposure and slowly enter the systemic circulation. The dermal absorption of a commercial PCB mixture was modeled, and the results suggest that the net result of the differences in absorbance rates would be a greater body burden of higher chlorinated PCBs relative to those that have a lower chlorine content.

Goffin V, Letawe C, Pierard GE. **Effect of organic solvents on normal human stratum corneum: evaluation by the corneoxenometry bioassay.** Dermatology 1997;195(4):321-4.

BACKGROUND: Organic solvents alter the stratum corneum structure and barrier function.

OBJECTIVE: To measure the effect of various solvents upon human stratum corneum using the ex vivo corneoxenometry bioassay which is a variant of corneoxenometry. METHODS: Corneoxenometry entails collection of human stratum corneum by cyanoacrylate. The material is immersed in organic solvents for periods ranging from 1 to 120 min. After staining the samples with a toluidine blue-basic fuchsin solution, the color is measured using reflectance colorimetry. Solvent aggressivity to the stratum corneum correlates with the color darkening of the samples. RESULTS: The least aggressive solvent was hexane, followed by ethanol, methanol, hexane-ethanol, chloroform, chloroform-methanol and hexane-methanol. The influence of contact time between solvents and the stratum corneum showed a

logarithmic pattern which varied according to the solvent. **CONCLUSION:** Data are in line with previous experiments conducted in vivo and in vitro, thus indicating the predictive value of corneoxenometry. Such a bioassay may avoid hazards of some in vivo human testings.

Henry F, Goffin V, Maibach HI, Pierard GE. **Regional differences in stratum corneum reactivity to surfactants. Quantitative assessment using the corneosurfametry bioassay.** Contact Dermatitis 1997;37(6):271-5.

The skin does not react similarly to the presence of xenobiotics over all anatomic sites. Distinct regional differences have been described for irritancy and percutaneous absorption. The present study assesses the regional variation of stratum corneum reactivity to surfactants using the corneosurfametry bioassay. Stratum corneum was harvested from 6 body sites in 20 young adults. Corneosurfametry was performed using water, 1% SLS and a 5% soap solution. Data show that the best variable to assess regional variability in irritancy is the overall difference in corneosurfametry (ODC), comparing the effect of a given surfactant with water. The dorsal hand and volar forearm were the least reactive, the neck, forehead, back and dorsal foot the most reactive, sites. It is concluded that the corneosurfametry bioassay, through the ODC variable, is a practically noninvasive tool for the evaluation of regional variation in irritancy at the level of the stratum corneum.

Hisoire G, Bucks D. **Unexpected finding in percutaneous absorption observed between haired and hairless guinea pig skin.** J Pharm Sci 1997 Mar;86:398-400.

IPA COPYRIGHT: ASHP To evaluate the percutaneous absorption and possible targeted follicular delivery of tretinoin (retinoic acid) in formulation with PPG-12/SMDI copolymer (polyolprepolymer-2), ethanolic gels with tretinoin alone and with 10% of the copolymer were prepared and evaluated for percutaneous absorption in haired and hairless guinea pigs. The hairless guinea pig skin was more permeable than haired guinea pig skin despite the lower follicular density in the hairless animal. Also, the addition of PPG-12/SMDI copolymer significantly decreased tretinoin penetration.

Ho HO, Chen LC, Lin HM, Sheu MT. **Penetration enhancement by menthol combined with a solubilization effect in a mixed solvent system.** J. Controlled Release 1998 Feb 2;51:301-11.

IPA COPYRIGHT: ASHP The improvement in the solubility of indomethacin due to the presence of different concentrations of menthol (0-12% w/v) in cosolvent systems consisting of different ratios of water, propylene glycol, and ethyl alcohol was assessed, and the percutaneous penetration of indomethacin through excised nude mouse (strain ICR) skin was studied using 3 of the cosolvent systems containing 0-12% w/v menthol that were able to solubilize concentrations of 1, 1.5, or 2% w/v of indomethacin. Menthol improved the solubility of indomethacin in the cosolvent systems to different extents. A cosolvent system with an equal ratio of all 3 solvents showed the greatest improvement in the solubility of indomethacin for all concentrations of menthol. The penetration rate of indomethacin through mouse skin was greatly enhanced by the addition of menthol to the cosolvent systems. Also, the penetration rate increased as the concentration of menthol in the cosolvent systems increased, reaching a maximum for a specific concentration of menthol in each cosolvent system.

Jadoul A, Preat V. **Electrically enhanced transdermal delivery of domperidone.** Int J Pharm 1997 Aug 26;154:229-34.

IPA COPYRIGHT: ASHP To investigate whether transdermal delivery of domperidone can be enhanced to therapeutic levels by iontophoresis and/or electroporation, in vitro studies using hairless rat skin were performed with a solution of domperidone in 9.5% ethyl alcohol (ethanol). Iontophoresis (2 h at 0.4 mA/cm²) increased the transdermal permeation by a factor of 15 as compared to passive diffusion. Application of 5 long high voltage pulses increased the domperidone permeation by a factor of up to 70. Application of 1 pulse prior to iontophoresis provided similar penetration enhancement to 5 pulses. No significant enhancement was provided by application of 1 short pulse prior to iontophoresis, probably due to a different mechanism of permeabilization and/or recovery kinetics to the initial permeability state. The domperidone permeation flux by skin electroporation was in the range of the fluxes measured with chemical penetration enhancers but the lag time was reduced. It was concluded that due to the low hydrosolubility of domperidone, electrically enhanced flux remains too low for therapeutic application.

Jiang R, Roberts MS, Prankerd RJ, Benson HA. **Percutaneous absorption of sunscreen agents from liquid paraffin: self-association of octyl salicylate and effects on skin flux.** J Pharm Sci 1997 Jul;86:791-6.

IPA COPYRIGHT: ASHP The percutaneous absorption of octyl salicylate from mineral oil (liquid paraffin), effects of octyl salicylate on skin permeability, and partitioning of octyl salicylate between mineral oil and aqueous receptor phases were studied using model membranes. Partition coefficients increased with an increase in octyl salicylate concentration. Self-association of octyl salicylate seemed to result in reduced activity of coefficient of the solute in mineral oil. Support for proposed self-association was found by infrared spectrometry and molecular models. No significant changes in permeability were found for skin or membrane filters except for the polyethylene membrane, in which a plasticization effect was seen at octyl salicylate concentration above 30%. The permeabilities of 2 membrane filters remained unchanged with a range of concentrations of octyl salicylate, indicating their suitability for future diffusion studies of sunscreen agents.

Johnson ME, Blankschtein D, Langer R. **Evaluation of solute permeation through the stratum corneum: lateral bilayer diffusion as the primary transport mechanism.** J Pharm Sci 1997 Oct;86:1162-72.

IPA COPYRIGHT: ASHP A mathematical model for the macroscopic transport of solutes across stratum corneum via the interkeratinocyte lipid domain was developed, the permeation of model solutes through cadaver skin was studied, and the lateral diffusion coefficients in stratum corneum lipid bilayers were calculated from permeation measurements and compared with reported values in stratum corneum extracted lipids. Good qualitative and quantitative agreement was observed. This indicated that diffusive resistance associated with lateral diffusion was sufficient to explain the overall resistance of solute permeation. Diffusive resistance associated with interfacial transbilayer transport was not capable of explaining experimental permeation, providing support for this finding. Lateral diffusion analysis also showed a bifunctional size dependence of transport within the stratum corneum, with a strong size dependence for small solutes (<300 Da) and weak size dependence for larger solutes.

Koschier FJ, Roth RN, Wallace KA, Curren RD, Harbell JW. **A comparison of three-dimensional human skin models to evaluate the dermal irritation of selected petroleum products.** In Vitro Toxicol 1997;10(4):391-405.

BIOSIS COPYRIGHT: BIOL ABS. The in vitro evaluation of the dermal irritation potential of volatile or water insoluble petroleum products has generally proved difficult. We attempted to overcome these difficulties by utilizing three commercially supplied human skin constructs to assess the dermal irritation potential of 14 petroleum refinery streams. Tissues were treated with the neat test materials for periods of up to 24 hours. Certain modifications to the standard dosing procedures were developed to address the solvent and volatile nature of the test materials. Four end points were assessed at each exposure time. Culture medium under the tissue was sampled for lactate dehydrogenase (LDH), interleukin-1-alpha (IL-1alpha), and prostaglandin E2 (PGE2) concentrations. The ET50 (time to reduce the MTT conversion to 50% of time matched controls) was calculated for each test material in each system. In general, the ET50 times for all three skin models ranked many of the 14 (10 for living skin equivalent (LSE)) test materials similarly. Spearman rank order analysis comparing the in vitro cytotoxicity data with the Primary Dental Irritation Index (PDII) scores gave values of 0.54 (LSE), 0.41 (ZK1300), and 0.79 (EpiDerm). The Actual ET50s of the more irritating materials varied between the three systems with the ZK1300 system appearing to show the greatest toxicity. LDH release in the three systems corroborated cytotoxicity estimated by the MTT50. Cytokine levels were normalized to the amount produced at the ET50 time point. These data indicated that IL-1alpha concentrations showed reasonable correlations with the known in vivo irritation level, especially in the EpiDerm cultures. It appeared that for all three models the best prediction of in vivo irritation came from a combination of cytotoxicity and IL-1alpha release measurements. Materials that had very low in vivo irritancy (PDII : 1.5) generally had MTT50 values of > 24 hours and released no measurable IL-1alpha. Materials with moderate in vivo irritancy levels (1.5 > PDII < 4.0) had MTT50 values of > 24 hours but did show some induction of IL-1alpha. Materials with higher irritancy (PDII : 4.0) generally caused both cytotoxicity and cytokine release.

Leung HW, Blaszcak DL. **The skin sensitization potential of four alkylalkanolamines.** *Vet Hum Toxicol* 1998;40(2):65-7.

Lewis D, Paulo M, Faustino E, Farinha A. **In vitro comparative studies of transdermal nicotine delivery systems.** *Int J Pharm* 1997 Mar 28;148:177-89.

IPA COPYRIGHT: ASHP The release rates of nicotine from 3 nicotine transdermal patch formulations (Nicotinell; Nicotrans; Nicodisc) were studied in vitro using recently proposed United States Pharmacopeia (USP) release tests and assays, and Franz diffusion cells with membranes of porcine ear or human skin. No significant differences were found between release profiles obtained by the different release test methods for each device. Total nicotine content could not be determined for 1 device at modest agitation speeds by methyl alcohol (methanol) extraction. Some simple approaches to comparing animal models and release tests with human skin experiments in vitro are discussed in relation to potential applications to quality control of transdermal delivery systems and other topical preparations.

Ogiso T, Paku T, Iwaki M, Tanino T. **Mechanism of the enhancement effect of n-octyl-beta-D-thioglucoside on the transdermal penetration of fluorescein isothiocyanate-labeled dextrans and the molecular weight dependence of water-soluble penetrants through stripped skin.** *J Pharm Sci* 1994 Dec;83:1676-81.

IPA COPYRIGHT: ASHP To investigate the mechanism of enhancing effect of n-octyl-beta-D-thioglucoside, the in vitro penetration of fluorescein isothiocyanate-labeled dextrans of molecular weight

4400-69,000 Da through hairless rat skin and stripped skin alone and with 1.5% n-octyl-beta-D-thioglucoside was evaluated; scanning electron micrographs were also evaluated. All of the dextrans penetrated more easily in the presence of n-octyl-beta-D-thioglucoside with high fluxes equivalent to those through stripped skin. n-Octyl-beta-D-thioglucoside significantly solubilized the stratum corneum proteins and ceramides during the initial time stage. Micrographs demonstrated that n-octyl-beta-D-thioglucoside treatment changed the cell membrane. It was concluded that the enhancement mechanisms of n-octyl-beta-D-thioglucoside may be different from other lipophilic enhancers.

Oh SY, Jeong SY, Park TG, Lee JH. **Enhanced transdermal delivery of AZT (zidovudine) using iontophoresis and penetration enhancer.** J Controlled Release 1998 Feb 12;51:161-8.

IPA COPYRIGHT: ASHP The effects of iontophoresis and the use of a mixture of 2 penetration enhancers, propylene glycol and oleic acid, in conjunction with iontophoresis, on the transdermal permeation of zidovudine (AZT) across excised hairless mouse skin were studied using solutions of the drug and drug loaded karaya gum matrices. Compared to passive diffusion, the iontophoretic flux of zidovudine from solutions of the drug was 5-40 fold greater, depending on the magnitude of current density. The iontophoretic flux of zidovudine from karaya gum matrices was slower than that from solutions of the drug. Incorporation of the mixture of penetration enhancers into karaya gum matrices markedly increased both the passive and iontophoretic flux of zidovudine.

Parnigotto PP, Bernuzzo S, Bruno P, Conconi MT, Montesi F. **Characterization and applications of human epidermis reconstructed in vitro on de-epidermized derma.** Farmaco 1998;53(2):125-31.

In recent years in vitro models have been developed to avoid the use of animals in cutaneous toxicological studies. Submerged human keratinocyte cultures in vitro could be so far employed as an alternative to animal testing and a good correlation between skin irritation and cytotoxicity has been demonstrated. Nevertheless, these submerged cultures are lacking in the stratum corneum which acts as a barrier to chemical toxicity, so that this type of culture is far from the in vivo situation. A better alternative method seems to be the use of in vitro reconstructed skin at the air-liquid interface that closely resembles the in vivo situation. In this work, in a first step we have characterized human epidermis reconstructed in vitro on de-epidermized derma (DED) after a two-week air exposure. Human skin reconstituted in vitro on DED was histologically similar to the in vivo skin. A stratified epidermis including the stratum corneum was obtained. The presence of basal lamina as well as of various important markers for epidermal differentiation (involucrin, K10 keratin, and filaggrin) were revealed. In a second step we have tested the cytotoxic and morphological effects of four surfactants on our model. A good rank correlation has been shown to exist between the irritation potency of surfactants on our model and reported ocular irritancy in vivo. From our results, in vitro reconstituted human skin could represent an attractive model for irritancy testing and could be an in vitro replacement for animal testing.

Pellet MA, Watkinson AC, Hadgraft J, Brain KR. **Comparison of permeability data from traditional diffusion cells and ATR-FTIR spectroscopy. Part 2. Determination of diffusional pathlengths in synthetic membranes and human stratum corneum.** Int J Pharm 1997 Aug 26;154:217-27.

IPA COPYRIGHT: ASHP The morphological structure of the inner and outer regions of human stratum corneum (S_C) were investigated using attenuated total reflectance Fourier transform infrared (ATR-

FTIR) spectroscopy; also, diffusional pathlengths in silicone membranes and human SC were determined using ATR-FTIR spectroscopic data and regular skin diffusion cell data. It was shown that diffusion coefficients for a model permeant, 4-cyanophenol, were lower in the more compact regions of the inner layers of the SC when compared to diffusion coefficients in the outer layers. Partition coefficients between SC and aqueous vehicles were higher in the outer layers than the inner layers. These data demonstrated a 4 fold lower permeability of skin to 4-cyanophenol in the inner layers relative to the outer layers of the SC. The combination of diffusion cell data and ATR-FTIR spectroscopic data was also used to determine diffusional pathlengths across synthetic silicone membranes and human SC. In all cases, the pathlengths were similar to the thickness of the membranes. It was concluded that morphological differences between the inner and outer regions of the SC are reflected in variations in permeability.

Pellett MA, Roberts MS, Hadgraft J. **Supersaturated solutions evaluated with an in vitro stratum corneum tape stripping technique.** Int J Pharm 1997 May 12;151:91-8.

IPA COPYRIGHT: ASHP The permeation of supersaturated solutions of piroxicam (1-4 degrees of saturation) across human skin in diffusion cells was studied using an in vitro stratum corneum tape stripping technique. Supersaturated solutions produced a linear relationship between the degree of saturation and the amount of piroxicam in the stratum corneum. The amount of drug in the viable layers of skin also increased with increasing degree of saturation. The enhanced penetration of drug from supersaturated solutions may have occurred as a result of the antinucleating ability of the intercellular lipids of the stratum corneum.

Robinson MK, Perkins MA, Basketter DA. **Application of a 4-h human patch test method for comparative and investigative assessment of skin irritation.** Contact Dermatitis 1998;38(4):194-202.

A human 4-h patch test has recently been developed for testing the irritation hazard potential of chemicals. The original method was developed for comparative irritation assessments relative to benchmark irritants using simple statistical tests. In this context, the method has been shown to be robust in intralaboratory testing over time. Recent interlaboratory testing has also established the consistency of the method in assessment of the relative irritation potential of selected chemicals. These data help to position the method as a suitable replacement for animal test methods in assessment of skin irritation hazard. In addition, the method has great utility for investigating different parameters of clinical skin irritation. Using kinetic response patterns and curve fitting analysis, we have compared the relative irritation potential of chemicals in greater detail, using as a basis the time required for test subjects to respond as well as the incidence of positive responses. Also, using the response to 20% sodium dodecyl sulfate (SDS) as a benchmark, we've been able to examine the intersubject variation in clinical skin irritation responses. In general, subjects most reactive to 20% SDS, in terms of the exposure time required to produce a positive response, were relatively more sensitive to a 2nd irritant chemical as well. However, this was not an absolute correlation in that some test subjects showed divergent patterns of response. The method was also used to compare directly the relative skin reactivity of different populations, based on race (Caucasian versus Asian) or on neurosensory skin sensitivity. Our results using this acute exposure test method indicate little difference in visually assessed skin irritation among these diverse human subpopulations.

Roper CS, Howes D, Blain PG, Williams FM. **A comparison of the absorption of a series of ethoxylates through rat skin in vitro.** *Toxicol In Vitro* 1998;12(1):57-65.

BIOSIS COPYRIGHT: BIOL ABS. The penetration of phenoxyethanol, dodecyl decaethoxylate and dodecyl monoethoxylate through rat skin was described in in vitro model systems using static cells with aqueous ethanol as receptor or flow-through diffusion cells with tissue culture medium as receptor. Absorption of 2-phenoxyethanol was similar in the static (64%) and flow-through diffusion cells (43%) suggesting that the choice of receptor fluid did not greatly influence prediction of penetration for this compound although the rate of absorption was higher in the static than flow-through cell, which reduced overall losses. A similar absorption was noted if evaporative losses were taken into consideration (static cell, 94%: flow-through diffusion cell, 85%). Dodecyl decaethoxylate absorption was influenced by the choice of receptor fluid; the absorption was 49.4% with aqueous ethanol, but was only 7% with tissue culture medium. However, using tissue culture medium as receptor, more material was located in the stratum corneum and skin than with aqueous ethanol. The highly lipophilic dodecyl monoethoxylate was rapidly absorbed into the lipophilic aqueous ethanol receptor fluid with total penetration of 102% at 8 hours. However, penetration to tissue culture medium was much lower in the flow-through diffusion cell. When stratum corneum, skin and receptor fluid were included in the estimate of penetration, total absorption was 33.3%, which increased to 66% when evaporative losses were considered. In conclusion, absorption profiles reflected the physicochemical properties of the ethoxylates, and the choice of receptor fluid appeared to be the most important factor in the choice of an in vitro model for predicting percutaneous absorption.

Roper CS, Howes D, Blain PG, Williams FM. **Percutaneous penetration of 2-phenoxyethanol through rat and human skin.** *Food Chem Toxicol* 1997;35(10-11):1009-16.

BIOSIS COPYRIGHT: BIOL ABS. 2-Phenoxyethanol applied in methanol was absorbed (64 : 4.4% at 24 hr) through unoccluded rat skin in vitro in the static diffusion cell with ethanol/water as receptor fluid. By comparison (43 : 3.7% in 24 hr) was absorbed in the flow-through diffusion system with tissue culture medium as receptor fluid. 2-Phenoxyethanol applied in methanol was absorbed (59.3 : 7.0% at 6 hr) through unoccluded human skin in vitro in the flow-through diffusion cell with tissue culture medium. With both unoccluded cells, 2-phenoxyethanol was lost by evaporation but occlusion of the static cell reduced evaporation and increased total absorption to 98.8 : 7.0%. Skin, post mitochondrial fraction, metabolized phenoxyethanol to phenoxyacetic acid at 5% of the rate for liver. Metabolism was inhibited by 1 mM pyrazole, suggesting involvement of alcohol dehydrogenase. However, first-pass metabolism of phenoxyethanol to phenoxyacetic acid was not detected during percutaneous penetration through viable rat skin in the flow-through system. First-pass metabolism in the skin does not therefore have an influence on systemic availability of dermally absorbed phenoxyethanol. These measures of phenoxyethanol absorption through rat and human skin in vitro agree well with those obtained previously in vivo.

Santi P, Volpato NM, Bettini R, Catellani PL, Massimo G, Colombo P. **Transdermal iontophoresis of salmon calcitonin can reproduce the hypocalcemic effect of intravenous administration.** *Farmaco* 1997;52(6-7):445-8.

Salmon calcitonin (sCt) was administered by transdermal iontophoresis in rabbits, using a new drug reservoir assembled directly on the skin, based on a dry disc containing sCt to be dissolved at the

application site. The hypocalcemic effect was taken as a measure of the pharmacodynamic response. In rabbits, the results obtained show that salmon calcitonin skin penetration by iontophoresis, using pulsatile current of 0.8 mA/cm² on a reservoir containing 100 IU/Kg of sCt, was governed by the quantity of electric charge applied, mimicking the hypocalcemic response of 10 IU/Kg intravenous administration.

Skov MJ, Quigley JW, Bucks DA. **Topical delivery system for tretinoin: research and clinical implications.** J Pharm Sci 1997;86(10):1138-43.

A novel topical tretinoin gel formulation containing a patented TopiCare Delivery Compound, polyolprepolymer-2 (PP-2), was shown to significantly reduce local irritation relative to a marketed tretinoin gel preparation while maintaining clinical efficacy in the treatment of acne. Several in vitro percutaneous absorption studies were conducted with 0.025% tretinoin as a model compound to determine the possible mechanism of action of PP-2 on drug delivery into and through human cadaver skin. Results of these studies have repeatedly shown that a new topical gel formulation containing PP-2 significantly reduces tretinoin penetration while potentially enhancing epidermal deposition compared with a commercial topical gel preparation at the same tretinoin concentration. These studies further support a mechanism of action whereby PP-2 serves as a retentate for drug delivery by formation of a liquid reservoir of polymer and solubilized drug on the skin surface and in the upper layers of the skin, thereby modifying delivery of tretinoin into and through skin. This reservoir of drug and polymer was established within 15 min after topical application, and tretinoin was shown to be highly associated with PP-2. These in vitro findings provide a model by which a new tretinoin gel formulation containing PP-2 reduces irritation relative to a commercial tretinoin gel while maintaining clinical efficacy in the treatment of acne vulgaris.

Stott PW, Williams AC, Barry BW. **Transdermal delivery from eutectic systems: enhanced permeation of a model drug, ibuprofen.** J Controlled Release 1998 Jan 2;50:297-308.

IPA COPYRIGHT: ASHP The formation of eutectic systems between ibuprofen and 7 terpene skin penetration enhancers was studied and, by using the eutectic systems as donors, the effects of melting point depression of the delivery system on transdermal delivery were investigated. Results indicated that only terpenes that formed hydrogen bonds with ibuprofen formed eutectic systems. The resultant melting point depression of the delivery system was correlated with a significant increase in transdermal permeation.

Turner NG, Ferry L, Price M, Cullander C, Guy RH. **Iontophoresis of poly-L-lysines: role of molecular weight?** Pharm Res 1997 Oct;14:1322-31.

IPA COPYRIGHT: ASHP A series of fluorescently labeled poly-L-lysines was used to study the extent and distribution of iontophoretic skin penetration as a function of molecular weight in vitro using laser scanning confocal microscopy. Relative to passive controls, iontophoresis greatly enhanced the penetration of the 4 KDa analog and slightly elevated the delivery of the 7 KDa compound, but had no effect on the transport of the 26 KDa polymer. Iontophoresis increased transport via follicular pathways only slightly more than that through nonfollicular pathways for the 4 KDa and 7 KDa polymer molecules.

Vives MA, Macian M, Seguer J, Infante MR, Vinardell MP. **Irritancy potential induced by surfactants derived from lysine.** Toxicol In Vitro 1997;11(6):779-83.

BIOSIS COPYRIGHT: BIOL ABS. The ocular irritancy potential of surfactants of the anionic and non-ionic type, derived from lysine has been tested by the hen's egg test-choriollantoic membrane (HET-CAM) to correlate the potential irritation with the structure of the surfactant, in order to synthesize the less irritant surfactant for their use in the pharmaceutical and cosmetics industry. The anionic compounds, independently of the counterion, showed an irritant action higher than non-ionic surfactants. Among the anionic surfactants the presence of lysine as cation reduced the degree of irritation; nevertheless, the salt of lysine of lauric acid was a severe irritant. The non-ionic surfactant with two chains was less irritant than the corresponding compound with one chain and represented the best compound for ocular application. The time of appearance of haemorrhage, vasoconstriction and coagulation is used to evaluate the degree of irritation. There was a close relationship between the concentration and the time of the appearance of vasoconstriction at the concentrations tested.

Wu PC, Huang YB, Fang JY, Tsai YH. **In vitro percutaneous absorption of captopril.** Int J Pharm 1997 Mar 14;148:41-6.

IPA COPYRIGHT: ASHP To evaluate the in vitro percutaneous absorption of captopril, 4 available skin membranes (mouse, rat, rabbit, pig) and human skin were utilized; the enhancing effect of penetration enhancers including fatty alcohols, aliphatic esters, and other compounds on captopril penetration through excised rabbit skin were also evaluated. The flux of captopril increased in the order of human=pig<rabbit<rat<mouse. The penetration rate of captopril through rabbit skin was optimal to evaluate the variation of formulations. Hence, the rabbit skin was picked out as a model membrane for in vitro penetration experiments of captopril. The flux of captopril was increased linearly when the concentration of captopril increased from 1% to 10%. The enhancement of fatty alcohols and aliphatic esters were related to the chain length of enhancers. The C6-C10 of fatty alcohols and n-butyl acetate (butyl acetate) were among the enhancers that showed the most enhancing effect on captopril penetration.

Yokomizo Y. **Effect of phosphatidylglycerol on the in vitro percutaneous drug penetration through the dorsal skin of guinea pigs, and analysis of the molecular mechanism, using (ATR-FTIR) spectroscopy.** Int J Pharm 1997 Feb 28;147:219-31.

IPA COPYRIGHT: ASHP The effects of phosphatidylglycerol on the percutaneous penetration of a water insoluble drug, tenoxicam, and a water soluble drug, diclofenac sodium, through the dorsal skin of guinea pigs were studied in vitro using attenuated total reflectance Fourier transform infrared spectroscopy. Tenoxicam penetration was dependent on phosphatidylglycerol concentration (up to 6%). The mechanism of the enhancing effect of phosphatidylglycerol on tenoxicam appeared to include an increase in the diffusion coefficient and partition coefficient and an increase in the fluidity of intercellular lipid domain. The effect of phosphatidylglycerol on diclofenac penetration appeared to involve an increase in the diffusion coefficient. The penetration of diclofenac was not dependent on phosphatidylglycerol concentration. Phosphatidylglycerol appeared to mainly affect the intercellular lipid pathway.

Baer KN, Goulden CE. **Evaluation of a high-hardness COMBO medium and frozen algae for *Daphnia magna***. *Ecotoxicol Environ Saf* 1998;39(3):201-6.

BIOSIS COPYRIGHT: BIOL ABS. A new high-hardness (H-H) COMBO medium for long-term culturing and bioassay testing of *Daphnia magna* was evaluated in 21-day survival, growth, and reproduction studies. The COMBO medium originally designed for softwater daphnid species (i.e., *D. pulex*) was modified for the hardwater species *D. magna*. This medium also allowed continuous culturing of a green algal food source for daphnids, *Ankistrodesmus falcatus*. After 12 generations of culturing in H-H COMBO medium, the mean fecundity was 128 : 12 (coefficient of variation=9.4%). No significant observations of dead young, aborted eggs, or reduced antennae were made during the 6 months of continuous culturing. The no-observable-effect concentration (NOEC) for a reference toxicant standard, 3,4-dichloroaniline (3,4-DCA), was 8.3 µg/liter, which is similar to published values. These studies demonstrate that H-H COMBO is an acceptable medium for use for culturing and bioassay testing with *D. magna*. Additional experiments using frozen aliquots of *A. falcatus* were conducted. Although mean fecundity (64 : 7) and adult length were significantly decreased from the values for daphnids fed fresh algae, the NOEC for 3,4-DCA was >25 µg/liter. The use of this alternative food source in bioassay testing appears promising, but further optimization of feeding rates is warranted.

Baganz D, Starks G, Steinberg C. **Impact of the cyanobacteria toxin, microcystin-LR on behaviour of zebrafish *Danio rerio***. *Water Res* 1998;32(3):948-52.

BIOSIS COPYRIGHT: BIOL ABS. Quantitative behaviour of zebrafish, *Danio rerio*, was recorded to assess and predict long-term sublethal effects of the cyanobacteria toxin microcystin-LR (MCYST-LR; nominal concentrations of 0.5, 5, 15 and 50 µg litre⁻¹) by using the automated BehavioQuantg. The exposure to MCYST-LR caused dose-effect related changes in spontaneous locomotor activity. Whereas the two lower exposure concentrations (0.5 and 5 µg litre⁻¹) caused an increase in daytime motility, elevated exposures led to significantly decreased motilities. The highest exposure (50 µg litre⁻¹) also reduced the spawning activity and success. In contrast to daytime activities, night-time swimming activity was significantly greater at the higher MCYST-LR exposures. The chronobiological analysis indicated a phase shift of maximum swimming activities and a lowered reaction on trigger points like feeding, at dusk and dawn. Furthermore, the results indicate some adverse consequences in reproduction success and in the spatial and temporal fit of the fish into its habitat.

Bruner MA, Rao M, Dumont JN, Hull M, Jones T, Bantle JA. **Ground and surface water developmental toxicity at a municipal landfill: Description and weather-related variation.**

Ecotoxicol Environ Saf 1998;39(3):215-26.

BIOSIS COPYRIGHT: BIOL ABS. Contaminated groundwater poses a significant health hazard and may also impact wildlife such as amphibians when it surfaces. Using FETAX (Frog Embryo Teratogenesis Assay-Xenopus), the developmental toxicity of ground and surface water samples near a closed municipal landfill at Norman, OK, were evaluated. The groundwater samples were taken from a network of wells in a shallow, unconfined aquifer downgradient from the landfill. Surface water samples were obtained from a pond and small stream adjacent to the landfill. Surface water samples from a reference site in similar habitat were also analyzed. Groundwater samples were highly toxic in the area near the landfill, indicating a plume of toxicants. Surface water samples from the landfill site

demonstrated elevated developmental toxicity. This toxicity was temporally variable and was significantly correlated with weather conditions during the 3 days prior to sampling. Mortality was negatively correlated with cumulative rain and relative humidity. Mortality was positively correlated with solar radiation and net radiation. No significant correlations were observed between mortality and weather parameters for days 4-7 preceding sampling.

Bureau R, Faucon JC, Faisant J, Briens F, Rault S. **Applicability of the free energies of solvation for the prediction of ecotoxicity: study of chlorophenols.** SAR QSAR Environ Res 1997;6(3-4):163-81. Free energies of solvation of chlorophenols were calculated in two solvents: water and n-hexadecane from the AMSOL program. These free energies of solvation are the sum of two terms: polarization free energies (ΔG_{ENP}) and cavity, dispersion, solvent structure free energies ($G^{\text{degree CDS}}$). This study shows, in the case of chlorophenols, that a direct relation exists between one of the two components for the calculation of free energy ($G^{\text{degree CDS}}$) in water and n-hexadecane, and the ecotoxicity values for five biological systems. We point out the interest of using these new descriptors in QSAR study.

Burkholder JM. **Implications of harmful microalgae and heterotrophic dinoflagellates in management of sustainable marine fisheries.** Ecol Appl 1998;8(1 Suppl):S37-S62.

BIOSIS COPYRIGHT: BIOL ABS. Worldwide increases in the frequency and spatial extent of blooms of harmful marine microalgae and heterotrophic dinoflagellates suggest that these species are becoming an increasingly important influence on year class strength of marine fishes through both direct and indirect mechanisms. Impacts on fish populations from harmful marine microalgae and heterotrophic dinoflagellates have been considered primarily from the limited view of acute or lethal influences. Accumulating evidence indicates that insidious sublethal and chronic impacts to both fish and human health from these organisms, such as long-term behavior alteration, increased susceptibility to cancers and other diseases, depressed feeding, and impaired reproduction, may be substantial and pervasive. For some harmful species, significant indirect impacts that promote critical habitat loss or disrupt the microbial food web balance also have been documented. Because successful models to predict the behavior and growth of most of these species have not yet been developed, and because toxins for many are poorly characterized, a clear anthropocentric focus has guided management strategies for confronting their outbreaks. The extent to which management takes the fundamental step of acknowledging scientifically demonstrated linkages among harmful microalgae, shellfish contamination, fish kills, and human health impacts has also been seriously constrained by political dictates stemming from economic considerations. Without federal involvement, and without catastrophe of human death or widescale serious human illness, little progress historically has been realized in the development of effective management strategies to mitigate lethal impacts to fish or other organisms. Many long-known taxa such as certain red tide dinoflagellates apparently can increase independently of human influences other than physical transport. However, some newly discovered toxic or otherwise harmful taxa have been correlated with cultural eutrophication in poorly flushed fish nursery grounds such as estuaries and coastal waters. Outbreaks of certain warm-optimal species have coincided with El Nino events, suggesting that warming trends in global climate change may stimulate their growth and extend or shift their range. The available information points to a critical need for a more proactive, concerted effort to determine the full range of chronic/sublethal effects, as well as acute impacts, on marine fish populations by harmful marine microalgae and heterotrophic dinoflagellates, so that their increasingly important

influence can be factored into reliable plans for sustainable fisheries management.

Ciarelli S, Vonck W A, Van Straalen N, Stronkhorst J. **Ecotoxicity assessment of contaminated dredged material with the marine amphipod *Corophium volutator***. Arch Environ Contam Toxicol 1998;34(4):350-6.

BIOSIS COPYRIGHT: BIOL ABS. The incorporation of toxicological data from bioassays can improve the present system of sediment quality criteria in the Netherlands. The use of acute lethality toxicity tests alone does not however provide sufficient discrimination and sensitivity for predicting ecological effects of slightly and moderately contaminated dredged material. Sublethal endpoints are needed for the assessment of environmental hazards of such dredged material. In this study, two approaches were used to identify toxicity of marine sediments collected from 16 locations classified as slightly and moderately contaminated on the basis of chemical data: (1) a comparison of growth vs. mortality as different endpoints in the marine amphipod *Corophium volutator* (Pallas); (2) an investigation on the use of sediment dilutions to characterize the degree of toxicity. The influence of sediment storage time on toxicity was also evaluated. In four out of 16 locations, mortality over 10 days of exposure ranged 80-100%; in two out of 16 locations mortality ranged 40-60%. In the other 10 locations, mortality was below 15%. Results on growth showed that in all locations final dry weight values were significantly lower (a factor of 1.5 to 3) than in controls. Results of dilution experiments showed that if sediments were diluted with a reference sediment of similar physicochemical characteristics, total concentrations of metals, mineral oil, and PAHs decreased as expected and so did the effects on *C. volutator*. In the 100% contaminated sediments growth was reduced by 32-60% compared to controls. The dilution rate necessary to reduce toxicity to the EC10 value for growth of *C. volutator* was considered an appropriate endpoint for the evaluation. When sediments were stored for a period of 3-5 months at 4°C and retested, effects on mortality and growth decreased, although some effects on growth were still measured after 5 months of storage. The experiments illustrate the usefulness of ecotoxicity assessment to evaluate contaminated dredged material.

Cima F, Ballarin L, Bressa G, Sabbadin A, Burighel P. **Triphenyltin pesticides in sea water as immunotoxins for tunicates**. Marine Chem 1997;58(3-4):267-73.

BIOSIS COPYRIGHT: BIOL ABS. Triphenyltin derivatives (TPTs, Fentin) cause a severe immunotoxicity in tunicates, having significant inhibitory effects on yeast phagocytosis by hemocytes of the colonial ascidian *Botryllus schlosseri* - TPTC > TPTA > TPTH - in a concentration-dependent manner. The same order of inhibition is observed for Ca²⁺-ATPase activity, suggesting that the inhibition of this enzyme and of phagocytosis may be closely linked. Addition of 20 µg/ml calmodulin is able to reverse the inhibition of Ca²⁺-ATPase activity by TPTs, but not to restore the phagocytosis index. These results support the hypothesis that mitochondrial oxidative phosphorylation may also be involved, as O₂- production is inhibited TPTA > TPTC > TPTH - in a concentration-dependent manner, but not restored by calmodulin. Besides, TPTs cause some morphological changes without cytolysis, suggesting an interaction with cytoskeletal components, whereas viability is not affected up to 100 µM for TPTA and TPTC, and 1 mM for TPTH.

Clare AS. **Towards nontoxic antifouling**. J Marine Biotechnol 1998;6(1):3-6.

BIOSIS COPYRIGHT: BIOL ABS. Environmental problems posed by metal-based antifouling coatings,

in particular those containing organotins, have led to legislation curbing their use and a concomitant increase in interest in the development of nontoxic alternatives. Two main avenues are being pursued: (1) the development of foul-release or easy-release coatings that interfere with the normal adhesion of marine organisms and (2) the isolation of marine natural products that inhibit settlement of potential epibionts on the host organism and accordingly could be incorporated into coatings to inhibit biofouling. This presentation will concentrate on the latter option, highlighting similarities in functional groups of some identified compounds and the testing of much simplified structural analogs. While natural products and analogs that exhibit broad spectrum activity at nontoxic concentrations have been identified, little is known about their mechanism of action. Such information could potentially be of use in screening for nontoxic antifoulants and in allowing the more rational design of analogs for testing. Studies are now underway that are attempting to unravel the cellular and molecular basis of settlement of several marine organisms, notably invertebrate larvae. Recent findings on signal transduction pathways involved in settlement of the economically important fouling barnacle, *Balanus amphitrite*, lend further credence to an earlier suggestion of a recurring theme in chemical signaling. A possible consequence of these molecular relationships is that effective broadspectrum natural product antifoulants could also affect nontarget species. However, natural products have existed for millions of years and bacteria responsible for their degradation have very likely evolved. The environmental problems posed by a natural product approach are, therefore, suggested to be low and could be further reduced by appropriate engineering of coatings.

Degen B, Scholz F. **Ecological genetics in forest ecosystems under stress as analysed by the simulation model ECO-GENE.** Chemosphere 1998;36(4-5):819-24.

BIOSIS COPYRIGHT: BIOL ABS. Previous investigations revealed that air pollutants influence the genetic structure of forest tree populations by affecting the genetic system via various single population genetic processes. A holistic approach that allows the integration of these results and an estimation of the importance of these affected processes including their interactions when operating together in the genetic system is missing. Therefore the simulation model ECO-GENE was developed. The model integrates the most important processes. The model is generic, it can be used for different research tasks (such as estimation of the effect of air pollutants, climate change, silviculture etc.) and serve as tool for decision making in different fields of application. One example for the model application is demonstrated. Here a good agreement between the empirically observed and the pertinent simulations for parameter sets of the mating system was found. These results indicate that the model assumptions on the components of the mating system and their interrelations are realistic.

Hanazato T. **Growth analysis of *Daphnia* early juvenile stages as an alternative method to test the chronic effect of chemicals.** Chemosphere 1998;36(8):1903-9.

BIOSIS COPYRIGHT: BIOL ABS. It is recognized that the mature size and clutch size of the cladoceran *Daphnia* are strongly affected by the size at birth and growth rate during the early juvenile stages. Individuals at the neonatal stage are most sensitive to environmental stress such as toxic chemicals, and therefore such stress may indirectly control the reproductivity of *Daphnia* by directly affecting the growth of neonates. These facts suggest that growth rate during the juvenile stages would be a good indicator of the effect of toxic chemicals on *Daphnia*'s reproduction, which is the parameter usually determined in chronic toxicity tests. In the present paper, I propose a new short-term test that

involved analysis of *Daphnia* growth at the early juvenile stages.

Hauser B, Schrader G, Bahadir M. **Comparison of acute toxicity and genotoxic concentrations of single compounds and waste elutriates using the Microtox/Mutatox test system.** *Ecotoxicol Environ Saf* 1997;38(3):227-31.

BIOSIS COPYRIGHT: BIOL ABS. The Mutatox test has been developed by Microbics Corp., U.S.A., in addition to the widely used acute toxicity test Microtox. The Mutatox test indicates the presence of any material in a test sample that causes genetic damage to dark variants of the luminescent bacteria *Vibrio fischeri*. As the Microtox test is less time-consuming and more cost-effective than the Mutatox test, the possibility of using the EC50 measured by Microtox for rangefinding of genotoxic concentrations for the Mutatox test was examined. Both tests were applied on single compounds and several waste elutriates. The genotoxic potential of two PAH metabolites-1-hydroxypyrene and 9-fluorenone-1-carboxylic acid-was detected. According to the present results the highest concentration of a sample in the Mutatox test should in general exceed the EC50 by about 5-10 times. Elutriates were submitted to analyses of TOC, heavy metals, phenols, and PAH; additionally GC/MS screening analyses were carried out. In most cases correlations of ecotoxicological effects with single contaminants were not possible, but it can be assumed that these effects were produced by the interaction of inorganic and organic compounds present in the elutriates.

Hudson LA, Ciborowski JJ. **Teratogenic and genotoxic responses of larval *Chironomus salinarius* group (Diptera: Chironomidae) to contaminated sediment.** *Environ Toxicol Chem* 1996 Aug;15(8):1375-81.

Chironomids are important indicators of the effects of sediment-bound contaminants. However, cause-effect relationships between contaminant exposure and stress-related indicators other than growth and survival have not been demonstrated. In the laboratory, we conducted 10-d exposures of *Chironomus salinarius* group Kieffer larvae to mixtures of contaminated (Trenton Channel, Detroit River, MI) sediment diluted with uncontaminated, formulated sediment (sand, sculptor's clay, and potting soil) in ratios of 1:0, 1:1, 1:3, 1:7, 1:15, and 0:1. Larvae were inoculated into either recently mixed sediments or those allowed to age for 7 d. Surviving larvae were examined for mouthpart (mentum) deformities. Giant chromosomes of the salivary glands were examined for reduction in relative nucleolus diameter (RND). Proportions of larvae with mentum deformities and of larvae with reduced RND increased linearly with each doubling of concentration of contaminated sediment. Deformities and RND reduction in individual larvae were independent responses. Incidences of deformities at low contamination doses were much greater for recently mixed sediments than for more aged sediments. Sediment age did not affect incidence of reduced RND except in the most contaminated treatment. Mentum deformities and reduced RND are quantifiable sublethal attributes reflecting stress responses to sediment contamination that can complement more traditional measures (survival and growth) in chironomid sediment bioassays.

Kosz-Vnenchak M, Rokosz K. **The comet assay for detection of potential genotoxicity of polluted water.** *Folia Biol* 1997;45(3-4):153-6.

BIOSIS COPYRIGHT: BIOL ABS. The aim of this study was to determine the potential genotoxic activity of polluted water samples taken from wastewater from selected industrial plants in Krakow: 1. the Thermal electric Power Station 2. the Institute of Metal Cutting. The recently developed single cell

gel assay (SCG or comet assay), which is a quick and simple technique for the evaluation of DNA damage and repair in individual cells, was used. The assay was carried out on human hepatoma cells (Hep G2) as target cells. A greater number of cells with comets was observed in those treated in vitro with the polluted water samples (70%-88%) than in those in the control (22%, 33%). These preliminary results indicate that comet assay can have an application in biomonitoring studies for determining the potential genotoxicity of water pollutants.

Lipscomb JC, Confer PD, Miller MR, Stamm SC, Snawder JE, Bandiera SM. **Metabolism of trichloroethylene and chloral hydrate by the Japanese medaka (*Oryzias latipes*) in vitro.** Environ Toxicol Chem 1998;17(2):325-32.

BIOSIS COPYRIGHT: BIOL ABS. Trichloroethylene (TRI), a common groundwater contaminant, is readily metabolized by mammals to produce chloral hydrate (CH), trichloroacetic acid (TCA), and trichloroethanol (TCOH). Cytochrome P450 (CYP) and other enzymes are responsible for formation of these metabolites, which are implicated in TRI's toxicity and carcinogenicity. To establish the validity of the Japanese medaka (*Oryzias latipes*) as an alternate test species for TRI, we examined the metabolism of TRI and CH, as well as CYP expression, in medaka liver preparations. Trichloroethylene was incubated with medaka microsomal protein, and metabolites were extracted and analyzed using gas chromatography. Microsome-mediated metabolism of TRI was observed, and a K_m value for TRI oxidation of 540 μM and a V_{max} value of 213 $\text{pmol}/\text{min mg}^{-1}$ protein were obtained. Conversion of TRI to CH, TCA, and TCOH was found with medaka hepatic subcellular fractions. In addition, a sex difference in hepatic microsomal TRI metabolism, specific CYP content, and ethoxyresorufin O-deethylase activity was noted. The lower specific activity of preparations from the livers of female medaka is compensated for by increased total protein in the larger liver mass of the female. Immunochemical analysis showed that CYP1A was readily detectable in medaka liver, but CYP2E1 was present at very low levels. These data suggest that TRI metabolism in medaka liver preparations mimics that observed in mammalian systems and supports their use as an alternative test species in the evaluation of the toxicity of TRI.

Mitchelmore CL, Birmelin C, Chipman JK, Livingstone DR. **Evidence for cytochrome P-450 catalysis and free radical involvement in the production of DNA strand breaks by benzo(a)pyrene and nitroaromatics in mussel (*Mytilus edulis* L.) digestive gland cells.** Aquatic Toxicol 1998;41(3):193-212.

BIOSIS COPYRIGHT: BIOL ABS. The mechanisms involved in the production of DNA strand breaks (SB) by model polycyclic aromatic hydrocarbon and nitroaromatic contaminants were investigated in isolated mussel (*Mytilus edulis* L.) digestive gland cell mixtures using the model compounds benzo(a)pyrene (BaP), 1-nitropyrene (1-NP) and nitrofurantoin (NF). Isolated cells were exposed in vitro to sub-cytotoxic concentrations (50 μM) of BaP, 1-NP or NF for 1 h in the dark at 15C in the absence or presence of various cytochrome P-450 inhibitors, antioxidant enzyme inhibitors, the free radical scavenger N-N-t-butyl-alpha-phenylnitron (PBN), and other modulators. DNA strand breakage was measured using the comet assay (SB results presented as % tail DNA and was significant for each genotoxicant at least $P < 0.05$). SB were seen for all three compounds and different metabolic pathways of genotoxicity were indicated for the three model compounds. BaP-induced strand breakage was indicated to be cytochrome P-450-catalysed and to occur via the production of BaP quinones because SB

were inhibited 94% by 50 μ M clotrimazole (inhibitor of digestive gland microsomal metabolism of BaP to quinones), stimulated 81% by 25 μ M dicoumarol (inhibitor of DT-diaphorase, EC 1.6.99.2, which metabolises quinones to hydroquinones) and unaffected by 50 μ M α -naphthoflavone (inhibitor of digestive gland microsomal metabolism of BaP to phenols and diols). Involvement of free radical(s) was indicated in BaP-induced strand breakage (75% SB inhibition by 50 μ M PBN), consistent with either BaP cation radical formation (i.e. 1-electron oxidation) and/or reactive oxygen species (ROS) generation via BaP quinone formation and redox cycling. 1-NP-induced SB was indicated to occur via free radical mechanism(s) (84% SB inhibition by 50 μ M PBN) and catalysis by different forms of cytochrome P-450 than for BaP (61% SB inhibition by 50 μ M α -naphthoflavone but none by clotrimazole). In contrast to BaP and 1-NP, NF induced strand breakage was indicated not to involve cytochrome P-450 (no SB inhibition by clotrimazole or α -naphthoflavone), but to involve free radical(s) (88% SB inhibition by 50 μ M PBN), consistent with redox cycling of NF and resultant DNA damage via superoxide anion radical ($O_2^{\cdot-}$) and other reactive oxygen species production. NF was more effective in producing SB compared to equimolar concentrations of BaP and 1-NP, possibly reflecting the greater direct redox cycling capacity of this compound.

Pacheco M, Santos MA. **Induction of EROD activity and genotoxic effects by polycyclic aromatic hydrocarbons and resin acids on the juvenile eel (*Anguilla anguilla* L.).** *Ecotoxicol Environ Saf* 1997;38(3):252-9.

BIOSIS COPYRIGHT: BIOL ABS. The purpose of this research is the study of total ethoxyresorufin O-deethylase (EROD) activity and erythrocytic nuclear abnormalities (NA) induction by polycyclic aromatic hydrocarbons (PAHs) and resin acids (RAs) on the juvenile eel *Anguilla anguilla* L. Juvenile eels were exposed, during 3 days, to the same molar concentration range (0, 0.1, 0.3, 0.9, and 2.7 μ M) of beta-naphthoflavone (BNF) and benzo(a)pyrene (BP)-PAHs and abietic acid (AA) and dehydroabietic acid (DHAA) RAs and one alkylating agent, such as cyclophosphamide (CP). Results revealed that both PAHs are able to significantly increase total EROD activity at 0.9 and 2.7 μ M, and both RAs at 0.3 μ M, whereas NA frequency was significantly increased by BP at 0.9 μ M and DHAA at 2.7 μ M. BNF was found to be the most potent EROD inducer, and BP exhibited the highest mutagenic potential. An additional experiment was carried out in order to study the BNF total EROD and NA induction over a period of 3, 6, and 9 days. An increased length of exposure, beyond 3 days, does not proportionally increase total EROD activity. However, 6 and 9 days BNF exposure significantly increases NA, reaching the (NA) levels previously determined for 3 days 0.9 μ M BP exposure.

Schmitz RP, Eisentraeger A, Lindvogt T, Moeller M, Dott W. **Increase of the toxic potential of synthetic ester lubricant oils by usage: application of aquatic bioassays and chemical analysis.** *Chemosphere* 1998;36(7):1513-22.

BIOSIS COPYRIGHT: BIOL ABS. The extent by which lubricant oils change their chemical composition and toxicological characteristics by usage is unknown. Risk classification is carried out only with unused fluids. Therefore, a series of lubricant oils based on synthetic esters was examined to characterize the toxic potential before and after an artificial aging process. The samples were examined by standardized bacterial growth assays and a luminescence inhibition test. The results demonstrate an increase of the toxicological potential after usage. A further chemical characterization by atomic

absorption spectroscopy (AAS), gas chromatography and mass spectrometry (GC-MS) revealed that volatile compounds like benzene, toluene, ethylbenzene and xylene are produced during the aging process. Heavy metals from catalysts and mechanical devices, are suspected to be partly responsible for the increase in toxicological potential. The alteration of toxicity suggests examination of the new as well as the used lubricant oil fluids for risk classification.

Shadrina LA. [**Ecotoxicological mapping of sea-shore waters by the bioassay method**]. *Gidrobiol Zh* 1997;33(6):50-5. (Rus)

BIOSIS COPYRIGHT: BIOL ABS. Results of toxicological monitoring of marine sea-shore waters by bioassay method and possibilities of its using in the ecotoxicological mapping are discussed.

Sjogren M. **Dispersal rates of Collembola in metal polluted soil**. *Pedobiologia* 1997;41(6):506-13. BIOSIS COPYRIGHT: BIOL ABS. Dispersal of soil animals may be affected by pollution. In this study the impact of heavy metals on dispersal rates of five collembolan species were estimated. The species differ in size, morphology and vertical distribution. Experiments were performed in plastic boxes filled with a 3 cm layer of more soil with 1 cm of litter on top. The soils were sampled at one heavily copper and zinc polluted site and one unpolluted site with similar vegetation and soil. Approximately 55 animals were introduced at one end of each box. Three different experimental set-ups were used: 1) unpolluted soil; 2) polluted soil; 3) unpolluted soil with a barrier of polluted soil in the middle. Two boxes of each set-up were destructively sampled after 1, 2, 3 and 4 weeks. All tested Collembola species had a higher rate of dispersal in polluted than in unpolluted soil. Dispersal rates were correlated to mean adult body size of the species. The polluted barrier in unpolluted soil did not change dispersal rates or result in a distribution differing from that in unpolluted soil. More specimens were recovered after 2-4 weeks of incubation in unpolluted than in polluted soil. Dispersal tests using soil animals inhabiting the topmost layers of the soil, and thus exposed to anthropogenic activities, could be an alternative to soil toxicity tests focussing on sublethal effects on survival and reproduction, but have to be further elaborated.

GENOTOXICITY AND MUTAGENESIS

. **Environmental Mutagen Society 29th Annual Meeting. Anaheim, California, USA. March 21-26, 1998. Abstracts.** *Environ Mol Mutagen* 1998;31(Suppl 29):1-87.

Ackermann EJ, Ang ET, Kanter JR, Tsigelny I, Taylor P. **Identification of pairwise interactions in the alpha-neurotoxin-nicotinic acetylcholine receptor complex through double mutant cycles.** *J Biol Chem* 1998;273(18):10958-64.

alpha-Neurotoxins are potent inhibitors of the nicotinic acetylcholine receptor (nAChR), binding with high affinity to the two agonist sites located on the extracellular domain. Previous site-directed mutagenesis had identified three residues on the alpha-neurotoxin from *Naja mossambica mossambica* (Lys27, Arg33, and Lys47) and four residues on the mouse muscle nAChR alpha-subunit (Val188, Tyr190, Pro197, and Asp200) as contributing to binding. In this study, thermodynamic mutant cycle analysis was applied to these sets of residues to identify specific pairwise interactions. Amino acid

variants of alpha-neurotoxin from *N. mossambica* at position 33 and of the nAChR at position 188 showed strong energetic couplings of 2-3 kcal/mol at both binding sites. Consistently smaller yet significant linkages of 1.6-2.1 kcal/mol were also observed between variants at position 27 on the toxin and position 188 on the receptor. Additionally, toxin residue 27 coupled to the receptor residues 190, 197, and 200 at the alphadelta binding site with observed coupling energies of 1.5-1.9 kcal/mol. No linkages were found between toxin residue Lys47 and the receptor residues studied here. These results provide direct evidence that the two conserved cationic residues Arg33 and Lys27, located on loop II of the toxin structure, are binding in close proximity to the alpha-subunit region between residues 188-200. The toxin residue Arg33 is closer to Val188, where it is likely stabilized by adjacent negative or aromatic residues on the receptor structure. Lys27 is positioned closer to Tyr190, Pro197, and Asp200, where it is likely stabilized through electrostatic interaction with Asp200 and/or cation/pi interactions with Tyr190.

Adam M, Loppes R. **Use of the ARG7 gene as an insertional mutagen to clone PHON24, a gene required for derepressible neutral phosphatase activity in *Chlamydomonas reinhardtii*.** *Mol Gen Genet* 1998;258(1-2):123-32.

In *Chlamydomonas reinhardtii*, transforming DNA apparently integrates at random locations in the nuclear genome and generates a high number of mutants by gene inactivation. Twenty-four *phoN* mutants lacking the derepressible neutral (DN) phosphatase activity were isolated following transformation of the *cw15arg7* strain with plasmid pASL harbouring the ARG7 gene encoding argininosuccinate lyase. In all mutants resulting from the transformation with linearised pASL, a functional ARG7 copy was found to be closely linked to a *phoN* mutation but additional ARG7 copies were present elsewhere in the genome. Of the 13 mutants submitted to allelism analysis, four were allelic or tightly linked to the previously isolated MNNG-induced *phoN* mutants (*phoN2*, *phoN3*, *phoN24*), the remaining mutants were distributed among seven additional loci. To learn more about the function of the genes involved in DN phosphatase production, we cloned PHON24 by plasmid rescue and screening of a wild-type genomic library. One clone complemented the *phoN24* mutation in cotransformation experiments, as did several subcloned fragments. In all *phoN24+* transformants, the DN phosphatase activity was 2-3 times lower than in the wild-type strain but about 10 times higher than in the untransformed control. In wild-type, PHON24 transcript accumulation was independent of inorganic phosphate deficiency. The transcripts were present in the MNNG-induced *phoN24* mutant but were lacking in the two insertional *phoN24* mutants. Insertional mutagenesis has thus permitted the isolation of novel mutants which were missing after induction with a chemical mutagen and the cloning of a gene which is probably involved in the regulation of the DN phosphatase.

Agrawal RC, Mehrotra NK. **Assessment of mutagenic potential of propoxur and its modulation by indole-3-carbinol.** *Food Chem Toxicol* 1997;35(10-11):1081-4.

Propoxur is a widely used dithiocarbamate pesticide. In the present set of investigations, mutagenicity of propoxur (in formulation) was studied using the micronucleus assay in bone marrow of Swiss mice. Single intraperitoneal (i.p.) administration of 25 mg/kg body weight dose of propoxur, which is a maximum tolerated dose (MTD), significantly induced the micronucleus formation in bone marrow cells after a 24- and 48-hr exposure. A half and a quarter of the MTD (12.5 and 6.25 mg/kg) were found ineffective to induce the micronuclei formation after 24- and 48-hr time periods by the i.p. route.

However, the PCE:NCE ratio was inhibited significantly with all the dose levels at both time periods. Oral administration of propoxur at different dose levels also induced micronuclei formation. A single application of 50 and 25 mg/kg dose levels of propoxur, which are MTD and 50% of MTD, also significantly induced micronuclei formation after 24- and 48-hr time periods in bone marrow cells of Swiss mice as compared with solvent control group, whereas a 12.5 mg/kg dose of propoxur was ineffective in inducing micronuclei formation. Single application of indole-3-carbinol (I3C), a glucobrassicin derivative present in cruciferous vegetables, significantly inhibited the propoxur-induced micronuclei formation when it was given at the dose level of 500 mg/kg body weight 48 hr before the single application of propoxur. Therefore, it seems that propoxur is mutagenic in the above test systems and I3C inhibited the mutagenicity of propoxur significantly.

Airaksinen A, Hovi T. **Modified base compositions at degenerate positions of a mutagenic oligonucleotide enhance randomness in site-saturation mutagenesis.** *Nucleic Acids Res* 1998;26(2):576-81.

Site-saturation mutagenesis, using degenerate oligonucleotide primers, is a frequently used method in introducing various mutations in a selected target codon. Oligonucleotides that are synthesized using equimolar concentrations of nucleoside phosphoramidites (dA, dC, dG, dT) in the positions to be saturated, result in a mutant population that is biased towards the original nucleotides. We found that this bias could be eliminated by modifying the concentrations of nucleoside phosphoramidites during the oligonucleotide synthesis. We synthesized eight degenerate oligonucleotides to saturate eight different codons, and sequenced a total of 344 mutagenized codons. In six of these eight oligonucleotides, we reduced to varying extents the concentrations of those nucleotides in the target positions that would form base pairs with the template. From the data, we analyzed the effects of different base compositions in the oligonucleotides when mutagenizing different codons, the influence of the positions of mismatches, and the significance of different non-Watson-Crick base pairs. Based on these results, we suggest levels to which different phosphoramidites should be reduced when synthesizing oligonucleotides for site-saturation mutagenesis.

Alpertunga B, Omurtag GZ, Ozmentese N. **Investigation about the genotoxic activities of some herbal teas used as folk medicine.** *Acta Pharm Turc* 1997;39(3):105-10.

Anderson D, Plewa MJ. **The International Comet Assay Workshop.** *Mutagenesis* 1998;13(1):67-73. The Comet (single cell gel electrophoresis) assay primarily measures DNA strand breakage in single cells (Singh et al., 1988). Briefly, cells are suspended in low melting point agarose on a microscope slide. The slides are put in lysing buffer to allow the DNA to unwind and then in electrophoresis buffer. During electrophoresis the broken DNA moves towards the anode forming a Comet tail, with the greater the extent of damage, the greater the tail. Assays can be conducted under neutral or alkaline (> pH 13) conditions. Double-strand breaks are measured under neutral conditions and single-strand breaks under alkaline conditions, where abasic sites and other alkali-labile sites or intermediates in base or nucleotide excision repair can also be detected. There are several good reviews concerning the assay (McKelvey-Martin et al., 1993; Fairbairn et al., 1995; Tice, 1995).

Anderson D, Yu TW, Dobrzynska MM, Ribas G, Marcos R. **Effects in the Comet assay of storage**

conditions on human blood. *Teratog Carcinog Mutagen* 1997;17(3):115-25.

The Comet assay is a rapid and sensitive method for analyzing single cells for DNA damage. Using human lymphocytes, the assay is particularly useful for human monitoring studies, as well as for in vitro genotoxicity testing of chemicals. In such studies, it is not always possible to collect and process matched samples on the same day as the blood is taken. It would be useful if some samples could be stored and examined at a different time, without loss of viability or other factors affecting responses. It is thus important to understand the effects of storage conditions on blood to be used in such studies and how exposure or treatment might modify such responses. In a joint study in two laboratories, blood was taken from various donors and stored under different conditions. It was examined on day 1 (day on which sample was taken) and days 2, 3, 4, 5, or 8 at room temperature, 4 degrees C, or -20 degrees C. Cells were treated after storage (from day 2 onward) with bleomycin (BLM) and ethylnitrosourea (ENU). The data were analyzed either by eye (classifying cells with different categories of damage) or by using a computerized image analysis system (Kinetic Imaging Ltd., Liverpool UK. Software Package Comet 3.0) where the tail moment, which is considered to be a sensitive measurement, has been analyzed. There was no loss of cell viability at 4 degrees C or room temperature up to 8 days when measured by trypan blue dye exclusion. Findings suggest that on days 1-4 for the untreated samples at room temperature or 4 degrees C there were no biologically meaningful changes in both the different categories of cell damage and tail moment data. In treated cultures up to day 4, either at room temperature or at 4 degrees C, responses were only minimally affected and changes were considered not to be of biological significance. However, there was slightly less variability between samples at 4 degrees C than at room temperature in one laboratory. The reverse was true in the other. This would suggest that samples can probably be stored up to day 4 at 4 degrees C or room temperature without any untoward effects. Provided samples can be processed within this 4-day time frame, it would not seem necessary to cryopreserve samples at -196 degrees C.

Ando H, Mishina M. **Efficient mutagenesis of zebrafish by a DNA cross-linking agent.** *Neurosci Lett* 1998;244(2):81-4.

We developed a novel procedure for efficient mutagenesis of zebrafish using a DNA cross-linking agent 4,5',8-trimethylpsoralen (TMP), which is known to frequently induce small deletions in *Escherichia coli* and *Caenorhabditis elegans*. A specific-locus test and pilot screenings indicated that the TMP mutagenesis procedure was efficient. To confirm the successful mutagenesis by TMP, we characterized mutants with selective impairments in the nervous system. The no tectal neuron mutation hindered the development of the tectal neurons, while the edawakare mutation resulted in the enhancement of the extension and branching of the peripheral axons of trigeminal ganglion and Rohon-Beard sensory neurons. These results suggest that the TMP mutagenesis will provide an efficient method to isolate and characterize zebrafish mutants at molecular level.

Arimoto-Kobayashi S, Harada N, Tokunaga R, Odo J, Hayatsu H. **Adsorption of mutagens to chlorophyllin-chitosan, an insoluble form of chlorophyllin.** *Mutat Res* 1997;381(2):243-9.

Chlorophyllin, a water soluble derivative of chlorophyll is known to suppress the mutagenic and carcinogenic actions of compounds having polycyclic structures, e.g. heterocyclic amines and aflatoxin B1. There is evidence that this suppressing effect arises, at least in part, by a complex formation between the porphyrin-like structure of chlorophyllin and the planar molecular surfaces of these compounds. We

report here that chlorophyllin can form an insoluble salt-like material when mixed with chitosan, a polyglucosamine, and that the solid chlorophyllin-chitosan thus prepared can efficiently trap polycyclic mutagenic compounds. The adsorbed polycyclic mutagens were elutable with buffers of acidic pH, but only to small extents. Chlorophyllin-chitosan may be expected to be useful as an intercepting agent against polycyclic mutagens and carcinogens.

Arimoto-Kobayashi S, Hayatsu H. **Formation of direct-acting mutagens from mixtures of N-nitrosomorpholine and carboxylates by UVA irradiation.** Environ Mol Mutagen 1998;31(2):163-8. Previously, we found that a directly mutagenic compound is produced from N-nitrosopiperidine (NPIP) in phosphate buffer on exposure to near-ultraviolet light (UVA) and we identified its structure as alpha-hydroxy-N-nitrosopiperidine phosphate ester. In the present study, we show that a similar photoactivation of an N-nitrosamine can take place with carboxylates in place of phosphate. When a neutral solution of a mixture of N-nitrosomorpholine (NMOR) and sodium acetate was irradiated with UVA, the solution became directly mutagenic towards *Salmonella typhimurium* TA1535. O6-Alkylguanine-DNA alkyltransferase-deficient strains of *S. typhimurium* showed remarkably higher mutagenesis responses to this mutagen than the proficient strains. Citrate, succinate, and several other biological carboxylates were also effective in producing the mutagens. Since a treatment of the NMOR plus acetate photoproduct with carboxylic ester hydrolase resulted in a loss of the mutagenicity, the active principle is suggested to be an acetate-esterified derivative of NMOR. The role of the esters as intermediates in the photomutagenesis of nitrosamines is discussed.

Ashby J, Tinwell H, Callander RD, Kimber I, Clay P, Galloway SM, Hill RB, Greenwood SK, Gaulden ME, Ferguson MJ, et al. **Thalidomide: lack of mutagenic activity across phyla and genetic endpoints.** Mutat Res 1997;396(1-2):45-64.

BIOSIS COPYRIGHT: BIOL ABS. The human and rabbit teratogen thalidomide has been tested for mutagenicity in a wide range of assays, ranging from bacterial gene mutation assays conducted in vitro to in vivo cytogenetic assays conducted using rabbits, and including a variety of human-derived tissues. Thalidomide was not mutagenic to 6 strains of *Salmonella* when tested both in the presence and absence of Aroclor-induced rat liver S9 mix. This inactivity was confirmed in strains TA98 and TA100 using a 1-h pre-incubation assay protocol with the same S9 mix (10% S9), and additionally, in strain TA98 using 3 concentrations of S9 (4%, 10% and 30% S9 in S9 mix). Thalidomide was not clastogenic either to cultured human lymphocytes (whole blood cultures, minus S9 mix) or to Chinese hamster ovary (CHO) cells treated in vitro. Further, no cytotoxicity was observed in purified human lymphocytes when exposed to thalidomide up to the limit of its solubility in the medium in the presence and absence of liver S9 from Aroclor-induced pregnant rabbit. The CHO assays were conducted without metabolic activation and in the presence of a variety of sources of auxiliary metabolic activation (PB/beta NP-induced rat liver S9 mix, pooled male and female human liver S9 mix, uninduced and Aroclor-induced pregnant rabbit liver S9 mix and foetal rabbit S9 mix). Thalidomide did not induce micronuclei in isolated human lymphocytes (minus S9 mix) and it was non-mutagenic to mouse lymphoma L5178Y TK +/- cells when tested to the limits of its solubility in the culture medium (:S9 mix). No indication of recombinogenic or clastogenic activity was observed for thalidomide when tested in *Drosophila*. In addition, it failed to induce chromosome aberrations in grasshopper neuroblasts when tested in the presence and absence of Aroclor-induced rat liver S9 mix. Some unusual chromosome morphologies

were observed in the grasshopper cytogenetic preparations indicating a potential of thalidomide to interact with chromosomal proteins. However, this potential was not evident in the human lymphocyte micronucleus assay, and thalidomide was apparently not reactive to the proteins of the mouse skin, as it gave negative results in a mouse local lymph node assay for skin sensitizing agents. Thalidomide was inactive in bone marrow micronucleus assays conducted using males and females from two strains of mice, and female New Zealand white rabbits. It is concluded that thalidomide is neither a mutagen nor an aneugen. This conclusion is discussed within the context of the results of earlier mutagenicity studies, the recent claim that thalidomide may be a heritable germ cell mutagen to humans, and the current interest in thalidomide for the treatment of immune system-related diseases.

Assmann N, Emmrich M, Kampf G, Kaiser M. **Genotoxic activity of important nitrobenzenes and nitroanilines in the Ames test and their structure-activity relationship.** *Mutat Res* 1997;395(2-3):139-44.

The most important commercially available nitro- and aminobenzenes and the explosive trinitrobenzene were tested for mutagenicity in the *Salmonella typhimurium* TA 98 and TA 100 both in the absence and presence of S 9. Ten of the 14 compounds tested (71%) were mutagenic. All the substances showed positive results in TA 98 and 4 substances were also mutagenic in TA 100. The three diaminobenzenes and 4-nitroaniline were mutagenic only with metabolic activation. All other compounds did not require the addition of S 9. Only nitrobenzene, 1,2-dinitrobenzene, aniline and 2-nitroaniline were negative in both strains. In summary, all substances that are derived from nitrobenzene or aniline by addition of a nitro group in the meta- or para-position were mutagenic, whereas nitrobenzene and aniline themselves and their ortho-derivates were nonmutagenic. The possible relationships between the position of the substituents and the mutagenicity are discussed.

Ballantyne B. **In vitro and in vivo genetic toxicology studies with bis(2-(dimethylamino)ethyl) ether.** *Toxic Subst Mech* 1998;17(1):57-79.

BIOSIS COPYRIGHT: BIOL ABS. Bis(2-(dimethylamino)ethyl) ether (DMAEE; CAS no. 3033-62-3), an industrial chemical, was investigated for potential genotoxicity by in vitro and in vivo tests. No mutagenic activity occurred in vitro in a *Salmonella typhimurium* reverse assay with strains TA98, TA100, TA1535, TA1537, and TA1538, or in a Chinese hamster ovary (CHO) cell forward gene mutation assay (HGPRT locus), with or without metabolic activation. In a CHO sister chromatid exchange (SCE) test, although weak activity was seen, there was no clear dose-response relationship, the effect was not replicated in duplicate cultures, and in the presence of metabolic activity statistical significance was only noted when the data from replicate cultures were combined. DMAEE did not stimulate unscheduled DNA synthesis in cultured rat hepatocytes, expressed as either nuclear or DNA-bound 3H-thymidine. A peripheral blood mouse micronucleus test (DMAEE at 45, 90, and 145 mg/kg, intraperitoneally) showed no increase in micronucleated polychromatophilic erythrocytes compared to a solvent control (water). Overall, DMAEE was not genotoxic, including the results from the test in vivo. The equivocal result with the SCE test may represent weak activity, or high sensitivity to the presence of a minor active component.

Ballantyne B. **In vitro genetic toxicology investigations with 5-ethylidene-2-norbornene.** *Toxic Subst Mech* 1998;17(2):133-51.

BIOSIS COPYRIGHT: BIOL ABS. 5-Ethylidene-2-norbornene (ENB, CAS No. 16219-75-3), an industrial chemical, was investigated for genotoxic potential with a battery of in vitro tests. No mutagenic activity occurred in the presence or absence of metabolic activation with a *Salmonella typhimurium* reverse assay with strains TA98, TA100, TA1535, TA1537, and TA1538, or in a Chinese hamster ovary (CHO) cell forward gene mutation assay (HGPRT locus). No effect was seen in a sister chromatid exchange test in CHO cells, with or without metabolic activation. A cytogenetic study, also conducted with CHO cells, did not show any increase in aberrant cells following dosing with ENB in the presence or absence of metabolic activation. The findings suggest an absence of a mutagenic or clastogenic potential for ENB.

Beier DR. **Zebrafish: genomics on the fast track.** *Genome Res* 1998;8(1):9-17.

Bila V, Kren V. **The teratogenic action of retinoic acid in rat congenic and recombinant inbred strains.** *Folia Biol (Praha)* 1996;42(4):167-73.

The teratogenic effect of all-trans retinoic acid (RA) was tested in the system of congenic and recombinant inbred (RI) strains of the laboratory rat carrying the mutant Lx allele which determines the polydactyly-luxate syndrome. It was demonstrated that the teratogenic effect of RA is influenced by both the genotype at the Lx locus and the modifying genes. The dose of 100 mg/kg administered by gavage on day 11 of pregnancy induced a statistically significant incidence of preaxial polydactyly in hind limbs of LEW/BN, +/Lx foetuses heterozygous in the mutant allele, whereas no specific limb defects were noted in foetuses LEW/BN, +/+ without the mutant allele. In foetuses homozygous in the mutant allele LEW/BN, Lx/Lx and SHR/BXH2, Lx/Lx, RA conversely induced a significant reduction in the number of toes on the preaxial side of hind limbs and a reduction of the zeugopodium, mostly the tibia. Reduction changes were more marked in SHR/BXH2, Lx/Lx foetuses, in which the tibia was entirely missing and sirenomelia was found. The foetuses in this group have in their genetic background a combination of modifiers that are responsible for oligodactyly and tibial hemimelia in the BXH2 strain. On the basis of continuing mapping of the rat genome, the testing of RA in the system of RI and congenic strains will be utilized for identification of so far hypothetical genes involved in morphogenesis.

Brennan RJ, Schiestl RH. **Diaminotoluenes induce intrachromosomal recombination and free radicals in *Saccharomyces cerevisiae*.** *Mutat Res* 1997;381(2):251-8.

The carcinogenicity of aniline-based aromatic amines is poorly reflected by their activity in short-term mutagenicity assays such as the *Salmonella typhimurium* reverse mutation (Ames) assay. More information about the mechanism of action of such carcinogens is needed. Here we report the effects on DEL recombination in *Saccharomyces cerevisiae* of the carcinogen 2,4-diaminotoluene and its structural isomer 2,6-diaminotoluene, which is reported to be non-carcinogenic. Both compounds are detected as equally mutagenic in the *Salmonella* assay. In the absence of any external metabolizing system both compounds were recombinagenic in the DEL assay with the carcinogen being a more potent inducer of deletions than the non-carcinogen. In the presence of Aroclor-induced rat liver S9, however, the carcinogen 2,4-diaminotoluene became a 2-fold more potent inducer of deletions, and the non-carcinogen 2,6-diaminotoluene was rendered less toxic and no induced recombination was observed. 2,4-Diaminotoluene is distinguished from its non-carcinogen analog in the DEL assay, therefore, on the

basis of a preferential activation of the carcinogen in the presence of a rat liver microsomal metabolizing system. Free radical species are produced by several carcinogens and have been implicated in carcinogenesis. We further investigated whether exposure of yeast to either 2,4-diaminotoluene or 2,6-diaminotoluene resulted in a rise in intracellular free radical species. The effects of the free radical scavenger N-acetylcysteine on toxicity and recombination induced by the two compounds and intracellular oxidation of the free radical-sensitive reporter compound dichlorofluorescein diacetate were studied. Both 2,4- and 2,6-diaminotoluene produced free radical species in yeast, indicating that the reason for the differential activity of the compounds for induced deletions is not reflected in any difference in the production of free radical species.

Broschard TH, Lebrun-Garcia A, Fuchs RP. **Mutagenic specificity of the food mutagen 2-amino-3-methylimidazo[4,5-f]quinoline in Escherichia coli using the yeast URA3 gene as a target.**

Carcinogenesis 1998;19(2):305-10.

2-Amino-3-methylimidazo[4,5-f]quinoline (IQ), a strong mutagen/carcinogen, belongs to a group of heterocyclic amines that are formed (ng/g amounts) during the cooking of protein containing food. The mutational specificity of IQ in Escherichia coli was determined in a forward mutation assay using the yeast URA3 gene as a target. The plasmid pTU-AC, containing the target URA3, was randomly modified in vitro using N-hydroxy-IQ, and subsequently transformed into an E. coli pyrF strain (DB6656). Mutant clones were directly selected by their ability to grow on medium containing 5-fluoro-orotic acid which is toxic to URA3+ clones and thereby selects for URA3- mutants. Single Strand Conformation Polymorphism (SSCP) was used to map the mutation-containing regions of URA3, so that it was necessary to sequence only the relevant, mutation-containing fragment and not the entire gene. At a modification level of 7 IQ-lesions/URA3 gene, the predominant mutations were base substitutions (approximately 70%), followed by complex gene rearrangements (approximately 20%) and frameshifts (approximately 10%). More than 96% of the base substitutions occurred at G:C base pairs and were predominantly G:C-->A:T transitions, followed by G:C-->T:A and G:C-->C:G transversions. Next neighbour analysis revealed that deoxyguanosines situated within the sequence 5'-TGC were more susceptible to mutations induced by IQ. With one exception, all frameshift mutations were -1 deletions at runs of three consecutive dGs. At higher IQ-modification levels, predominantly complex sequence rearrangements were observed.

Busca R, Martinez M, Vilella E, Peinado J, Gelpi JL, Deeb S, Auwerx J, Reina M, Vilaro S. **The carboxy-terminal region of human lipoprotein lipase is necessary for its exit from the endoplasmic reticulum.** J Lipid Res 1998;39(4):821-33.

Certain missense substitutions on the human lipase (hLPL) gene produce mutated proteins that are retained in different compartments along the secretory pathway. The purpose of the present study was to elucidate whether the C-terminal domain of the hLPL molecule could be important for secretion. We constructed by site-directed mutagenesis three carboxy-terminal mutated (F388-->Stop, K428-->Stop and K441-->Stop) hLPL cDNAs that were expressed in COS1 cells. Immunoblotting of cell extracts showed that all three constructs led to similar levels of protein. Both wild type (WT) hLPL and the truncated K441-->Stop hLPL were secreted to the extracellular medium, and presented a similar intracellular distribution pattern as shown by immunofluorescence. Neither F388-->Stop nor K428-->Stop hLPL₄₈ protein was detected in cell medium. Immunofluorescence experiments showed that both

truncated hLPL were retained within an intracellular compartment, which became larger. Double immunofluorescence analysis using antibodies against LPL and antiprotein disulfide isomerase as a marker showed that the truncated K428-->Stop hLPL was retained within the rough endoplasmic reticulum. This truncated protein was not found in other compartments in the secretory pathway, such as Golgi complex and lysosomes, indicating that it did not exit the endoplasmic reticulum. Further analysis of the C-terminal region of the LPL molecular model showed both that F388-->Stop and K428-->Stop hLPL truncated proteins are highly hydrophobic. As retention of secretory proteins in the rough endoplasmic reticulum is a quality control mechanism of the secretory pathway, we conclude that the C-terminal domain of hLPL is critical for correct intracellular processing of the newly synthesized protein.

Cadet J, Douki T, Ravanat JL. **Artifacts associated with the measurement of oxidized DNA bases.** Environ Health Perspect 1997;105(10):1034-9.

BIOSIS COPYRIGHT: BIOL ABS. In this paper we review recent aspects of the measurement of oxidized DNA bases, currently a matter of debate. There has long been an interest in the determination of the level of oxidized bases in cellular DNA under both normal and oxidative stress conditions. In this respect, the situation is confusing because variations that may be as large as two orders of magnitude have been reported for the yield of the formation of 8-oxo-7,8-dihydroguanine (8-oxoGua) in similar DNA samples. However, recent findings clearly show that application of several assays like gas chromatography-mass spectrometry (GC-MS) and (32P)-postlabeling may lead to a significant overestimation of the level of oxidized bases in cellular DNA. In particular, the silylation step, which is required to make the samples volatile for the GC-MS analysis, has been shown to induce oxidation of normal bases at the level of about one oxidized base per 10⁴ normal bases. This has been found to be a general process that applies in particular to 8-oxoGua, 8-oxo-7,8-dihydroadenine, 5-hydroxycytosine, 5-(hydroxymethyl)uracil, and 5-formyluracil. Interestingly, prepurification of the oxidized bases from DNA hydrolysate prior to the derivatization reaction prevents artifactual oxidation. Under these conditions, the level of oxidized bases measured by GC-MS is similar to that obtained by HPLC associated with electrochemical detection (HPLC-EC). It should be added that the level of 8-oxo-7,8-dihydro-2'-deoxyguanosine in control cellular DNA has been found to be about fivefold lower than in earlier HPLC-EC measurements by using appropriate conditions of extraction and enzymatic digestion of DNA. Similar conclusions were reached by measuring formamidopyrimidine-DNA glycosylase sensitive sites as revealed by the single cell gel electrophoresis (comet) assay.

Carmellino ML, Pagani G, Terreni M, Pregnotato M, Borgna P, Pastoni F, Zani F. **Antimicrobial and genotoxic activities of N-hydroxyalkyl-1, 2-benzisothiazol-3(2H)-one carbamic esters.** Farmaco 1997;52(6-7):359-66.

N-Hydroxyethyl- and N-hydroxypropyl-1,2-benzisothiazol-3(2H)-one carbamic esters were prepared in order to test their activity against representative bacterial and fungal strains. The obtained results were compared with those reported for parent alcohols and some interesting considerations were drawn. None of the studied derivatives possess genotoxic activity in the Bacillus subtilis rec-assay and Salmonella-microsome test.

Chen JX, Zheng Y, West M, Tang MS. **Carcinogens preferentially bind at methylated CpG in the p53 mutational hot spots.** Cancer Res 1998;58(10):2070-5.

The major mutational hot spots in human cancers occur at CpG sequences in the p53 gene. It is generally presumed that the majority of mutations at these sites result from the endogenous deamination of methylated cytosine. Using a UvrABC incision method, we have found that cytosine methylation greatly enhances guanine alkylation at all CpG sites in the p53 gene by a variety of carcinogens, including benzo(a)pyrene diol epoxide, benzo(g)chrysene diol epoxide, aflatoxin B1 8,9-epoxide, and N-acetoxy-2-acetylaminofluorene. These findings suggest that mutational hot spots at methylated CpG sequences in the p53 gene may be a consequence of preferential carcinogen binding at these sites.

Chiapella C, Moreno JA, Radovan RD, Gaubert N, Llagostera M. **Activation of arylamines to mutagenic product(s) by two in vitro plant systems.** *Mutat Res* 1997;394(1-3):45-51.

Plant activation of three isomers of phenylenediamine o- m- and p-phenylenediamine, has been studied. Two in vitro plant systems have been used: *Persea americana* S117 with mixed-function oxidase (MFO) and peroxidase activities, and *Zea mays* S9 which contains only peroxidase activity. As genetic endpoint, the classical *Salmonella* tester strains, TA98 and TA100, their derivatives with high O-acetyltransferase levels (YG1024 and YG1029, respectively) and TA98/1.8-DNP6, deficient in this enzyme, have been assayed. Of the three isomers studied, only m-PDA was activated to mutagenic product(s) by both plant systems. This activation required the bacterial O-acetyltransferase activity to give frameshift mutagenic product(s), detected in TA98 and YG1024 strains. In all the assays the *P. americana* system was more potent than the *Z. mays* system in activating m-PDA. A slight increase of the number of YG1029 revertants was detected when m-PDA was activated by *P. americana*, suggesting that this compound can be also converted into ultimate mutagenic product(s) that induce base-pair substitutions. m-PDA activation by *Z. mays* was dependent on the peroxidase activity of this system, but the activation produced by *P. americana* was totally dependent on MFOs, because a total inhibition of the mutagenic response was found when these activities were inhibited. In addition, the *P. americana* system was more potent in generating proximal mutagenic forms from m-PDA than S9 from non-induced rat liver, although S9 from Aroclor 1254-induced Sprague-Dawley male rats was the most potent system in the m-PDA activation. These results indicate that the *P. americana* system can be useful in determining the role of mixed-function oxidases in plant activation of xenobiotics.

Coia G, Ayres A, Lilley GG, Hudson PJ, Irving RA. **Use of mutator cells as a means for increasing production levels of a recombinant antibody directed against Hepatitis B.** *Gene* 1997;201(1-2):203-9.

A mutation strategy which utilises phage display technology and the *Escherichia coli* mutator strains, mutD5-FIT and XL1-RED, was applied to a Hepatitis B (HepB) specific single-chain Fv (scFv) to incorporate random mutations throughout the gene. Messenger RNA from a hybridoma producing antibodies against HepB was isolated, reverse transcribed and used as template for the production of scFv. Following production of the scFv protein using an *E. coli* expression vector (pGC), the scFv gene was recloned into a phage display vector (pHFA). This gene construct was introduced into *E. coli* mutator cells and the transformed cells were used as an inoculum for liquid cultures. After five cycles of growth at 37 degrees C, each followed by dilution and re-inoculation of fresh media, recombinant phage were recovered. Nucleotide sequence analysis of the scFv gene in phage selected on HBsAg-coated magnetic beads identified amino acid substitutions which produced an increase of greater than 10-fold in apparent production levels. Competitive ELISA studies showed that the selected scFv mutants appeared

to have similar affinity to HBsAg as the parent scFv. The apparent increase in production was not the result of improved surface characteristics of regions uniquely exposed in scFvs, as the sites did not correlate with the variable/constant interface of the scFv variable region normally masked in Fabs or IgGs.

Colvin ME, Seidl ET, Nielsen IM, Le Bui L, Hatch FT. **Deprotonation and hydride shifts in nitrenium and iminium forms of aminoimidazole-azaarene mutagens.** Chem Biol Interact 1997;108 (1-2):39-66.

The mutagenicity of many 2-aminoimidazole-azaarenes (AIA) is thought to be mediated by the nitrenium form of the exocyclic amine. This hypothesis is supported by the numerous correlations found between calculated and experimentally-measured chemical properties for the nitreniums and the mutagenic potencies of the nitreniums and their parent amines. One factor favoring high mutagenic potency is the presence of a methyl substituent in the 1- or 3-imidazole position. In this paper, we investigate both the deprotonation of the imidazole ring nitrogens in non-N-methylated AIA mutagens and the plausibility of a chemical pathway involving a 1-4 hydride shift to form an iminium ion, thereby stabilizing the cationic N-methyl substituted AIA mutagens. It has been widely noted that factors that stabilize the nitrenium moiety lead to significantly higher mutagenic potency; hence, the transformation of the nitrenium to a more stable species might be expected to increase the potency, provided that it does not eliminate the electrophilic reactivity of the compound. Using ab initio quantum chemistry and polarizable continuum solvation models, we find that the imidazole ring nitrogens of the nitrenium ions are extremely acidic. This suggests that upon formation of the exocyclic nitrenium these sites will deprotonate to form a neutral imine. We have also studied the 1-4 hydride shift from an imidazole ring methyl to the exocyclic nitrenium to form an iminium. We predict that for AIA mutagens with just two fused rings the resulting iminium species are more stable in the gas phase than the corresponding nitreniums. For mutagens with larger conjugated systems, the nitrenium is stabilized by resonance and is more stable than the corresponding iminium. In the aqueous phase, however, the iminium form is predicted to be more stable than the nitreniums for all polycyclic compounds studied. Although equilibrium calculations favor the iminium form, these have been experimentally shown to be short-lived and their actual concentration will depend on the complex kinetics of AIA mutagen metabolism. The quantum chemical results also show a strong correlation between the relative iminium-nitrenium energy difference and the charge on the exocyclic nitrogen.

De Flora S, Camoirano A, Cartiglia C, Ferguson L. **Modulation of the potency of promutagens and direct acting mutagens in bacteria by inhibitors of the multidrug resistance mechanism.** Mutagenesis 1997;12(6):431-5.

Multiple drug resistance (MDR) mechanisms are known to limit the effectiveness of some cancer chemotherapies, probably through enhancing P-glycoprotein-mediated drug efflux from mammalian cells. Similar mechanisms appear to act in other organisms, including bacteria, and may affect not only the toxicity but also the mutagenicity of certain chemicals. At least in some experimental situations, MDR can be overcome through concomitant treatment of the cells with various types of inhibitors. Two MDR inhibitors, verapamil, a calcium channel blocker, and trifluoperazine, a calmodulin inhibitor, were assayed for their ability to modulate the potency of nine mutagens with varying mechanisms of action in various *Salmonella typhimurium* his- strains. Neither verapamil nor trifluoperazine affected the direct

mutagenicity of sodium dichromate and 2-methoxy-6-chloro-9[3-(2-chloroethyl)amino-propyl-amino] dihydrochloride (ICR 191) or the S9-mediated mutagenicity of benzo[a]pyrene and 2-amino-3,4-dimethyl-amidazo[4,5-f]quinoline (MeIQ). Both modulators enhanced the direct mutagenicity of doxorubicin. Moreover, trifluoperazine sharply increased the S9-mediated mutagenicity of cyclophosphamide and 2-aminofluorene, while it consistently decreased the mutagenicity of 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2). The contrasting effect towards the aromatic amine 2-aminofluorene and the heterocyclic amine Trp-P-2, representative of important chemical families responsible for the bacterial mutagenicity of cigarette smoke, may explain the observed lack of influence of trifluoperazine on the mutagenicity of a cigarette smoke condensate. These observations extend the known range of chemical types whose mutagenicity can be modulated by inhibitors of MDR and suggest that there may be value in adding MDR inhibitors, especially trifluoperazine, to optimize the detection of mutagenicity by certain types of chemicals in the Salmonella/mammalian microsome mutagenicity test.

Diehl SR, Erickson RP. **Genome scan for teratogen-induced clefting susceptibility loci in the mouse: evidence of both allelic and locus heterogeneity distinguishing cleft lip and cleft palate.** Proc Natl Acad Sci U S A 1997 May 13;94(10):5231-6.

Nonsyndromic clefting of the lip and palate in humans has a highly complex etiology, with both multiple genetic loci and exposure to teratogens influencing susceptibility. Previous studies using mouse models have examined only very small portions of the genome. Here we report the findings of a genome-wide search for susceptibility genes for teratogen-induced clefting in the AXB and BXA set of recombinant inbred mouse strains. We compare results obtained using phenytoin (which induces cleft lip) and 6-aminonicotinamide (which induces cleft palate). We use a new statistical approach based on logistic regression suitable for these categorical data to identify several chromosomal regions as possible locations of clefting susceptibility loci, and we review candidate genes located within each region. Because cleft lip and cleft palate do not frequently co-aggregate in human families and because these structures arise semi-independently during development, these disorders are usually considered to be distinct in etiology. Our data, however, implicate several of the same chromosomal regions for both forms of clefting when teratogen-induced. Furthermore, different parental strain alleles are usually associated with clefting of the lip versus that of the palate (i.e., allelic heterogeneity). Because several other chromosomal regions are associated with only one form of clefting, locus heterogeneity also appears to be involved. Our findings in this mouse model suggest several priority areas for evaluation in human epidemiological studies.

Dillon CT, Lay PA, Bonin AM, Cholewa M, Legge GJ, Collins TJ, Kostka KL. **Permeability, cytotoxicity, and genotoxicity of chromium (V) and chromium (VI) complexes in V79 Chinese hamster lung cells.** Chem Res Toxicol 1998;11(2):119-29.

The genotoxicity of Cr(V) complexes in mammalian cells (V79 Chinese hamster lung cells) has been studied for the first time using the in vitro micronucleus assay. Two complexes were investigated, [CrO(ehba)₂]⁻, which undergoes ligand-exchange and disproportionation reactions in the cell growth medium, and [CrO(mampa)]⁻, which is chemically inert in the medium for the duration of the exposure period. Results of in vitro micronucleus assays show that both complexes are genotoxic and exhibit similar potencies to that of [Cr₂O₇]²⁻. The permeabilities of the Cr(V) complexes were also investigated

for the first time using particle-induced X-ray emission (PIXE) analysis of individual cells. The Cr uptake increased in the order: $[\text{Cr}(\text{phen})_2(\text{H}_2\text{O})_2]^{3+} < [\text{CrO}(\text{ehba})_2]^- < [\text{CrO}(\text{mampa})]^- < [\text{Cr}_2\text{O}_7]^{2-}$. Clonal assays showed that Cr(VI) exhibits an expectedly higher cytotoxicity than the Cr(V) complexes. While the genotoxicities of the Cr(V) and Cr(VI) complexes increase according to their permeabilities, the genotoxicities of the Cr(V) complexes are equal to, if not greater than, that of Cr(VI) in terms of the amount of Cr entering the cell. This supports other evidence that Cr(V), produced as a metabolic intermediate from the intracellular reduction of Cr(VI), may be important in Cr-induced cancers.

Duffaud F, Orsiere T, Digue L, Favre R, Botta A. [**Applicability to the detection of a genotoxic event of the human lymphocytes cytokinesis-block micronucleus test in cancer patients**]. Bull Cancer 1998;85(3):267-71. (Fre)

BIOSIS COPYRIGHT: BIOL ABS. The cytokinesis-blocked micronuclease assay (CBMN) is a short-term mutagenesis test which offers an easier and Less tedious alternative to metaphase chromosome analysis, with the advantage that exposure to both clastogens and aneugens may be detected. The CBMN assay has been used in evaluating the genotoxic consequences of exposures to environmental and occupational mutagens and carcinogens. Micronucleated cell rates (MN cell rates) were assessed in cytokinesis-blocked lymphocytes of 70 male and female cancer patients prior to any anticancer treatment. The study of interindividual variation factors showed that only age significantly affect MN cell rate, whereas sex, tobacco, alcohol, imaging techniques and tumour stage had no significant effect. The comparison of micronucleated cell rates in 198 healthy subjects and 70 cancer patients matched for age and sex showed a statistically significant difference. Spontaneous elevated MN cell rates of cancer patients refer to previous exposition of genotoxic or mutagenic environmental agents. Moreover, the MN cell rates in cancer patients most probably refers to various cellular lesions and genetic damages.

Fadool JM, Hartl DL, Dowling JE. **Transposition of the mariner element from *Drosophila mauritiana* in zebrafish**. Proc Natl Acad Sci U S A 1998;95(9):5182-6.

With the increased popularity of zebrafish (*Danio rerio*) for mutagenesis studies, efficient methods for manipulation of its genome are needed. One approach is the use of a transposable element as a vector for gene transfer in this species. We report here the transformation of zebrafish and germ-line transmission of the mariner element from *Drosophila mauritiana*. The mariner element was selected because its transposition is independent of host-specific factors. One- to two-cell-stage zebrafish embryos were coinjected with a supercoiled plasmid carrying the nonautonomous mariner element peach and mRNA encoding the transposase. Surviving larvae were reared to adulthood, and the transmission of peach to the F1 generation was tested by PCR. Four of the 12 founders, following plasmid injections on 2 different days, transmitted the element to their progeny. Inheritance of the transgene from the F1 to the F2 generation showed a Mendelian pattern. No plasmid sequences were detected by PCR or Southern blot analysis, indicating transposition of peach rather than random integration of the plasmid DNA. These data provide evidence of transformation of a vertebrate with a transposable element and support the host-independent mechanism for transposition of the mariner element. We suggest this system could be used for insertional mutagenesis or for identifying active regions of the genome in the zebrafish.

Ferguson LR, Ford JH. **Overlap between mutagens and teratogens**. Mutat Res 1998;396(1-2):1-8.

BIOSIS COPYRIGHT: BIOL ABS. RRM EDITORIAL HUMAN ANIMAL MUTAGENS

TERATOGENS TERATOGENESIS DNA XENOBIOTICS TOXICOLOGY MUTAGENESIS.

Frei H, Wurgler FE. **The vicinal chloroalcohols 1,3-dichloro-2-propanol (DC2P), 3-chloro-1,2-propanediol (3CPD) and 2-chloro-1,3-propanediol (2CPD) are not genotoxic in vivo in the wing spot test of *Drosophila melanogaster*.** *Mutat Res* 1997;394(1-3):59-68.

In this study, the vicinal chloroalcohols 1,3-dichloro-2-propanol (DC2P), 3-chloro-1,2-propanediol (3CPD) and 2-chloro-1,3-propanediol (2CPD) were investigated for genotoxicity in the wing spot test of *Drosophila*. DC2P is an important starting material in many processes of synthesis in chemical industry. 3CPD as well as some related glycerol chlorohydrins were identified in protein hydrolysates industrially used for the production of food items such as seasonings, sauces and soups. The wing spot test is a somatic mutation and recombination test (SMART) and is a sensitive in vivo assay for the detection of mutagens and promutagens. The test was applied here in its standard version with normal bioactivation and in a variant with increased cytochrome P450-dependent bioactivation capacity. All three compounds were clearly non-genotoxic in these in vivo assays. The results are in agreement with recent findings which strongly suggest that positive genotoxicity results in in vitro testing of vicinal chloroalcohols such as DC2P are due to directly acting genotoxic intermediates arising from a chemical reaction with the culture medium rather than from enzymatic biotransformation.

Henderson L, Wolfreys A, Fedyk J, Bourner C, Windebank S. **The ability of the Comet assay to discriminate between genotoxins and cytotoxins.** *Mutagenesis* 1998;13(1):89-94.

The Comet assay has been used widely in genetic toxicology, radiation biology and medical and environmental research. This assay detects single-strand breaks and alkali-labile sites in DNA and DNA degradation due to necrosis or apoptosis. It may also be modified to detect DNA cross-linking. Although a considerable number of chemicals have been tested in the assay there are many aspects of validation to be considered before the method could be considered to provide definitive evidence of genotoxic potential. For example, very few non-genotoxins have been tested to assess specificity of the Comet assay and there has been only one reported study which investigated whether the in vitro Comet assay is prone to false positive responses due to cytotoxicity. We have investigated the response of the alkaline Comet assay in TK6 human lymphoblastoid cells to cytotoxic damage and genotoxic damage. Several compounds which are toxic by different mechanisms were tested in the assay. Cycloheximide and trypsin gave a negative comet response at a highest dose of 5 mg/ml and no toxicity was observed. Sodium lauryl sulphate and potassium cyanide produced a significant increase in DNA migration at cell survival levels of $< \text{or} = 75\%$. The distribution of damaged cells indicated that cells at various stages of necrotic cell death were present. Hydrogen peroxide, 4-nitroquinoline oxide, 9-aminoacridine, ethyl methanesulphonate, N-nitroso-N-ethylurea and glyoxal gave a positive comet response. Mitomycin C was negative at survival levels of approximately 70%. These results indicate that the maximum concentration of test substance tested should produce viabilities $> 75\%$ in order to avoid false positive responses due to cytotoxicity. The assay was able to detect DNA damage induced by an alkylating agent, an intercalating agent and oxidative damage. The cross-linking agent mitomycin C was not detected if a cut-off point of 75% viability is used as the criterion of a positive response.

Henry B, Grant SG, Klopman G, Rosenkranz HS. **Induction of forward mutations at the thymidine kinase locus of mouse lymphoma cells: evidence for electrophilic and non-electrophilic**

mechanisms. *Mutat Res* 1998;397(2):313-35.

A database of 209 chemicals tested for induction of forward mutations at the heterozygous thymidine kinase (TK +/-) locus in L5178Y mouse lymphoma cells was analyzed for structure-activity relationships using the MultiCASE expert system. Consistent with evidence of several contributing biological mechanisms, these studies suggest that such mutations may occur by more than one mechanism. As might be expected, there was evidence for a component involving direct electrophilic attack on the cellular DNA, in a manner previously established as causative in the induction of mutations in *Salmonella*. In addition, however, there was also strong evidence for another mechanism or mechanisms involving chromosome missegregation, cellular toxicity or an alternate site of action, such as the microtubules.

Il'inskaia ON, Ivanchenko OB, Trubnikova GG, Valimukhametova DA, Tarasova RI, Bagautdinova DB. **[Genotoxic effects of metabolic derivatives of the new drug phosphabenzide]**. *Genetika* 1998;34(2):308-12. (Rus)

Genotoxic action of four possible metabolites of the new tranquilizer phosphabenzide (acetylphosphabenzide, diphenylphosphinylacetic acid, phosphabenzide hydrazone with pyruvic acid, bis-1,2-(diphenylphosphinylacetyl)hydrazine) has been studied. These metabolites belong to slightly toxic phosphororganic compounds. The Ames *Salmonella*/microsomes tests performed on strains TA100 and TA98 showed that of these compounds only acetylphosphabenzide possessed mutagenic action. Metabolic activation of liver microsomes decreased the mutagenic effect. The mechanism of action of acetylphosphabenzide is likely to involve the formation of acetylhydrazine, capable of producing active electrophiles attacking DNA.

Isaenko OA, Shvartsman PY. **[Heat shock modifies the teratogenic effect of griseofulvin in *Drosophila melanogaster*]**. *Genetika* 1995;31(4):583-5. (Rus)

BIOSIS COPYRIGHT: BIOL ABS. Modification of the teratogenic effect of griseofulvin by hyperthermia was demonstrated in line Canton-S of *Drosophila melanogaster*. Heat shock (37C for 45 min) during II instar larvae significantly decreased the frequency of chemomorphoses that phenocopy eye mutation (eyeless 4: 2.0).

Josephy PD, Evans DH, Parikh A, Guengerich FP. **Metabolic activation of aromatic amine mutagens by simultaneous expression of human cytochrome P450 1A2, NADPH-cytochrome P450 reductase, and N-acetyltransferase in *Escherichia coli***. *Chem Res Toxicol* 1998;11(1):70-4.

We describe the construction of a new strain of *Escherichia coli* designed to bioactivate aromatic amines and to detect their mutagenicity with high sensitivity. Strain DJ4309 bears two plasmids, a pACYC184-derived plasmid which expresses *Salmonella typhimurium* acetyl CoA:arylamine N-acetyltransferase (NAT) and a pBR322-derived plasmid which expresses human cytochrome P450 1A2 and NADPH-cytochrome P450 reductase. The combined actions of these enzymes convert aromatic amines into reactive, mutagenic N-acetoxy esters. The strain also carries a mutated copy of the lacZ gene (on an F' factor) which reverts to the wild-type gene by a -(GpC) frameshift mutation. Strain DJ4309 expresses high levels of NAT and cytochrome P450 1A2 and is very sensitive to mutagenesis induced by representative aromatic amines. Mutagenicity of 2-aminoanthracene in strain DJ4309 is higher than can be obtained by rat liver homogenate 9000g supernatant (S9) activation in the parent strain lacking the

P450 expression vector. Strain DJ4309 provides a useful system for detecting mutagenic aromatic amines and for studying their metabolism by human P450 1A2.

Justus T, Thomas SM. **Construction of a umuC'-luxAB plasmid for the detection of mutagenic DNA repair via luminescence.** *Mutat Res* 1998;398(1-2):131-41.

This paper describes a novel system for the detection of mutagenic DNA repair in *Escherichia coli*. The DNA damage inducible umuC gene of *Escherichia coli* has been fused to the luxAB genes from *Vibrio harveyi* that encode the enzyme luciferase. Mutagenicity has been assessed semi-quantitatively by the induction of bioluminescence. This system is simple, rapid and equivalent in sensitivity to other currently available test procedures. Its use in the detection of known SOS mutagens MMS, MNNG and UV is described.

Ko JY, Lui LT, Sheen TS, Lou PJ, Hsu MM. **Increased mutagen sensitivity in patients with head and neck cancer is less pronounced in patients with nasopharyngeal carcinoma.** *Arch Otolaryngol Head Neck Surg* 1998;124(5):578-81.

BACKGROUND: Mutagen sensitivity tested with bleomycin sulfate can determine a susceptible phenotype, which is relevant only in organs and tissues that have direct contact with the external environment. Patients with head and neck cancers have more mutagen sensitivity than control subjects without cancer, and the hypersensitive phenotype has a risk for the development of a second primary cancer. Head and neck cancers, however, represent a heterogeneous group of neoplasm. The biological behavior of nasopharyngeal carcinoma (NPC) and other head and neck cancers differs. **OBJECTIVE:** To evaluate the difference in mutagen sensitivity among patients without cancer, patients with NPC, patients with oral or oropharyngeal cancer (ORC), and patients with laryngeal or hypopharyngeal cancer (LHC). **DESIGN:** Peripheral blood was cultured at 37 degrees C, using 5% carbon dioxide, for 72 hours. After 67 hours of incubation, bleomycin in a concentration of 30 IU/L was added to induce chromatid breaks. The number of chromatid breaks per cell was scored in 50 metaphases of cultured lymphocytes and compared in the 4 groups. **SUBJECTS:** Patients with histologically proven squamous cell carcinoma of the mucosa of the upper digestive tract, which included 3 groups: patients with NPC, patients with ORC, and those with LHC. Control subjects were hospital inpatients with no tumor history. There were 35 patients in each group. **RESULTS:** The mean (+/-SD) number of breaks per cell in the control group and in the groups with NPC, ORC, and LHC were 0.80 (+/-0.32), 1.03 (+/-0.45), 1.30 (+/-0.44), and 1.35 (+/-0.46), respectively. All the cancer groups had significantly higher mean breaks per cell and a higher prevalence of hypersensitivity than the control group. Patients with NPC had a significantly lower mean number of breaks per cell than the group with ORC or that with LHC. **CONCLUSIONS:** Patients with NPC had less mutagen sensitivity than those with ORC or LHC. Our results support the clinical and epidemiological findings of a difference between NPC and other head and neck cancers. Environmental factors might play a less pronounced role in the carcinogenesis of NPC.

Koreshkova SV, Tanirbergenov TB, Tarasov VA. **[Prospects of SOS chromotest U.S.A.ge in predicting the carcinogenic activity of chemical compounds].** *Genetika* 1995;31(6):861-4. (Rus)

BIOSIS COPYRIGHT: BIOL ABS. When studying the carcinogenic activity of a compound, one of the basic problems is to determine the predictability of the methods used for testing; in our opinion, the most promising method is the SOS chromotest. To evaluate the test, we sampled 25 substances with a known

carcinogenic activity, which had not been tested with the SOS chromotest before. Properties of the SOS chromotest were analyzed on the basis of a database containing 154 substances at present, which are characterized with regard to the presence or absence of a carcinogenic effect in rodents. The results are distributed as follows: 121 carcinogens, of which 79 positively respond to the SOS chromotest; 33 noncarcinogens, of which 28 negatively respond to the SOS chromotest. The sensitivity and specificity of the SOS chromotest were measured as 65.3 and 84.9%, respectively. Comparing the results obtained with the Ames test and with the SOS chromotest, it was shown that the tests were similar in sensitivity and specificity. A similar predictability of both methods was also recorded.

Lalonde RT, Bu L, Henwood A, Fiumano J, Zhang L. **Bromine-, chlorine-, and mixed halogen-substituted 4-methyl-2(5H)-furanones: synthesis and mutagenic effects of halogen and hydroxyl group replacements.** Chem Res Toxicol 1997;10(12):1427-36.

The versatility of 4-(hydroxymethyl)-2(5H)-furanone as a starting point for the synthesis of several bromine and mixed halogen analogues of the potent water mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) has been demonstrated. However, in some preparations the yields of desired products were lower for bromine- than chlorine-substituted counterparts. A total of 12 bromine-, chlorine-, and mixed halogen-substituted 4-methyl-2(5H)-furanones were tested repeatedly in 10 independent experiments for levels of *Salmonella typhimurium* (TA100) mutagenicities. The purpose of these experiments was to determine the mutagenic response to changing halogen content, type, and position as well as to learn the measure of these responses in the presence and absence of the C-5 OH group. Mutagenicities reached levels of 10(3) and 10(2) rev/nmol for all trihalo- and dihalo-4-methyl-5-hydroxy-2(5H)-furanones, respectively, notwithstanding substitutions by bromine or chlorine. Trihalides lacking the C-5 hydroxyl group possessed mutagenicities of the order of 10(2) rev/nmol, while hydroxyl group absence in the dihalides resulted in potency levels of slightly less than 10 rev/nmol. Pairwise comparisons of compound mutagenicities showed that overall the C-5 H-by-OH replacement and, next in importance, increasing the number of C-6 halogens from one to two resulted in the greatest enhancements of mutagenicities. However, in comparing compound pairs within two different sets of four di- and trihalides, it was observed that replacement of a C-5 H by OH enhanced mutagenicity more for the dihalides than the trihalides indicating that increasing the C-6 halogen number simultaneously with replacing C-5 H by OH results in a nonlinear, additive enhancement. For fewer than half of the compound pairs compared, changing the C-6 halogen from chlorine to bromine resulted in small increases in mutagenicity, and for the remaining compound pairs, no increase could be discerned. This result points to the relative unimportance of only C-6 halogen type as a determinant of mutagenicity. Similarly, no impact on mutagenicity was observed for changing only the halogen type attached to C-3.

Lavrukhin OV, Lloyd RS. **Mutagenic replication in a human cell extract of DNAs containing site-specific and stereospecific benzo(a)pyrene-7,8-diol-9,10-epoxide DNA adducts placed on the leading and lagging strands.** Cancer Res 1998;58(5):887-91.

The environmental carcinogen benzo(a)pyrene-7,8-diol-9,10-epoxide (BPDE) forms DNA adducts with unique stereochemistries that may have divergent biological fates, depending on how they are processed within a cell. To investigate the effect of DNA bulky adduct stereoisomerism on the mutagenic outcome of translesion DNA replication in a human cell extract, oligonucleotides were synthesized that contained (+)- and (-)-anti-cis-BPDE enantiomers on N6 adenine at position 2 of the human N-ras 61 codon. Both

the nonadducted and BPDE-adducted oligonucleotides were introduced into two double-stranded vectors, replicative forms M13mp2SVoriL and M13mp2SVoriR, which contain SV40 origins of replication in two different orientations relative to the adduct insertion site. Nonadducted and adduct-containing vector DNA constructs were replicated in HeLa cytoplasmic extracts and then screened in bacteria for base substitutions at the adduct site. The mutation frequencies for the adducted DNAs were at least 10 times higher than for the nonadducted DNA and ranged from 5.5×10^{-4} to 1.5×10^{-3} . The (-)-anti-cis enantiomer was more than twice as mutagenic as the (+)-enantiomer. All three possible base mutations were present, with the A→G being the predominant one. No dramatic differences in replication fidelity were found when the adducts were placed on the leading versus the lagging strand of the vector.

Le Page F, Guy A, Cadet J, Sarasin A, Gentil A. **Repair and mutagenic potency of 8-oxoG:A and 8-oxoG:C base pairs in mammalian cells.** *Nucleic Acids Res* 1998;26(5):1276-81.

Replication of the oxidative lesion 8-oxo-7,8-dihydroguanine (GO) leads to the formation of both 8-oxo-7,8-dihydroguanine:adenine (GO:A) and 8-oxo-7,8-dihydroguanine:cytosine (GO:C) pairs. The repair and mutagenic potency of these two kinds of base pairs were studied in simian COS7 and human MRC5V1 cells using the shuttle vector technology. Shuttle vectors carrying a unique GO residue opposite either a C or an A were constructed, then transfected into recipient mammalian cells. DNA repair resulting in G:C pairs and mutation frequency, were determined using resistance to digestion by the Ngo MI restriction enzyme for screening and DNA sequencing of suspect mutants. Results showed that the GO:C mismatch was well repaired since almost no mutations were detected in the plasmid progeny obtained 72 h after cell transfection. The GO:A pair was poorly repaired since only 32-34% of the plasmid progeny contained G:C whereas two thirds contained A:T at the original site. Repair kinetics measured with a non-replicating vector deleted by 13 bp at the SV40 replication origin, showed that GO:A was slowly repaired. Only 30% of the mispairs were corrected in 12 h. During this time 100% of the plasmids containing GO:A pairs were replicated as seen by the replication kinetics in a vector with an intact SV40 replication origin. These results show that, under our experimental conditions, replication is occurring before completion of DNA repair which explains the high mutagenic potency of the GO:A mispair.

Li J, Ayyadevera R, Shmookler Reis RJ. **Carcinogens stimulate intrachromosomal homologous recombination at an endogenous locus in human diploid fibroblasts.** *Mutat Res* 1997;385(3):173-93.

Malyapa RS, Ahern EW, Straube WL, Moros EG, Pickard WF, Roti Roti JI. **Measurement of DNA damage after exposure to electromagnetic radiation in the cellular phone communication frequency band (835.62 and 847.74 MHz).** *Radiat Res* 1997;148(6):618-27.

BIOSIS COPYRIGHT: BIOL ABS. Mouse C3H 10T1/2 fibroblasts and human glioblastoma U87MG cells were exposed to cellular phone communication frequency radiations to investigate whether such exposure produces DNA damage in in vitro cultures. Two types of frequency modulations were studied: frequency-modulated continuous-wave (FMCW), with a carrier frequency of 835.62 MHz, and code-division multiple-access (CDMA) centered on 847.74 MHz. Exponentially growing (U87MG and C3H 10T1/2 cells) and plateau-phase (C3H 10T1/2 cells) cultures were exposed to either FMCW or CDMA radiation for varying periods up to 24 h in specially designed radial transmission lines (RTLs) that

provided relatively uniform exposure with a specific absorption rate (SAR) of 0.6 W/kg. Temperatures in the RTLs were monitored continuously and maintained at $37 \pm 0.3^\circ\text{C}$. Sham exposure of cultures in an RTL (negative control) and ^{137}Cs gamma-irradiated samples (positive control) were included with every experiment. The alkaline comet assay as described by Olive et al. (Exp. Cell Res. 198, 259-269, 1992) was used to measure DNA damage. No significant differences were observed between the test group exposed to FMCW or CDMA radiation and the sham-treated negative controls. Our results indicate that exposure of cultured mammalian cells to cellular phone communication frequencies under these conditions at an SAR of 0.6 W/kg does not cause DNA damage as measured by the alkaline comet assay.

Malyapa RS, Bi C, Ahern EW, Roti Roti JL. **Detection of DNA damage by the alkaline comet assay after exposure to low-dose gamma radiation.** Radiat Res 1998;149(4):396-400.

The alkaline comet assay as described by Olive et al. (Exp. Cell Res. 198, 259-267, 1992) was used to detect DNA damage in cells exposed to low doses (0-5 cGy) of gamma radiation. Experiments were performed using lymphocytes isolated from whole blood of rats. The comet parameters, normalized comet moment and comet length, described by Kent et al. (Int. J. Radiat. Biol. 67, 655-660, 1995), were used as measurements of DNA damage. It was observed that the alkaline comet assay can detect DNA damage at doses as low as 0.6 cGy. The results of the experiments using low-dose gamma radiation are comparable with published results obtained using the alkaline comet assay according to the method of Singh et al. (Int. J. Radiat. Biol. 66, 23-28, 1994). Based on this observation and analysis of results published previously, we conclude that the version of the alkaline comet assay described by Olive et al. is as sensitive as other modifications of the comet assay reported in literature for the detection of DNA damage in cells exposed to low doses of ionizing radiation.

Marczewska J, Koziorowska J. **Comparison of the induction of SOS repair in Escherichia coli PQ37 and PQ243 by antineoplastic agents.** Acta Pol Pharm 1997;54(1):35-41.

Six alkylating antineoplastic drugs (Cyclophosphamide, Chlorambucil, Busulfan, Melphalan, Streptozotocin and Lomustine) and two reference compounds (methyl methanesulphonate and N-methyl-N'-nitro-N-nitrosoguanidine) were investigated in the SOS Chromotest using the Escherichia coli strain PQ37 (wild-type) and derived strain (PQ243), which carries the same markers as PQ37 and additionally tagA alkA. As a measure of the SOS induced activity induction factors of *sflA::lacZ* expression were determined. The strain PQ243 was more sensitive towards all compounds inducing SOS DNA repair than the strain PQ37. Cyclophosphamide was detected as negative in the strain PQ243 in the presence of an exogenous metabolic activation system. Lomustine was inactive both in the mutant strain and in the wild-type strain in the presence of S9 mix fraction as well as in the absence of it. Melphalan and Busulfan (without or with S9 mix) were shown to be positive exclusively in the strain PQ243. Based on these results, we discuss the usefulness of the strain PQ243 in the monitoring of the genotoxicity of drugs and in the genetic analysis of their mode of action.

Miller B, Poetter-Locher F, Seelbach A, Stopper H, Utesch D, Madle S. **Evaluation of the in vitro micronucleus test as an alternative to the in vitro chromosomal aberration assay: position of the GUM working group on the in vitro micronucleus test.** Mutat Res 1998;410(1):81-116.

BIOSIS COPYRIGHT: BIOL ABS. In order to license a pharmaceutical or chemical, a compound has to

be tested for several genotoxicity endpoints, including the induction of chromosomal aberrations in vitro. A working group within the GUM has evaluated published data on the in vitro micronucleus test with the aim of judging its suitability as a replacement for the in vitro chromosomal aberration test. After strict rejection criteria were applied, a database including 96 publications and 34 compounds was obtained. For 30 of these compounds, data on both tests were available. For 24 of the 30, concordant results in both test systems were obtained (80% correlation). The discordant results in 6 compounds can be explained by a known or suspected aneugenic potential of these compounds. Considering that cell types and test protocols were extremely heterogeneous, this correlation is rather encouraging. Comparison of the different protocols, and experience established within the working group yielded several recommendations for the routine use of the in vitro micronucleus test. Although many cell lines are suitable, those most often used in genotoxicity testing (e.g. CHL, CHO, V79, human lymphocytes, L5178Y mouse lymphoma cells) are recommended. Cytochalasin B may be used in the case of human lymphocytes; however, the possibility of its interaction with aneugenic test compounds should be considered. For continuously dividing cell lines, cytochalasin B is not recommended by the working group. Although, there seems to be flexibility in the choice of treatment and sampling times, the average generation time of the chosen cell line of choice should be taken into account when determining sampling time, and treatment of cells for at least one cell cycle duration is recommended. The use of appropriate cytotoxicity tests is strongly recommended. Although studies on some parameters of the test protocol may be useful, the introduction of the in vitro micronucleus test into genotoxicity testing and guidelines should not be delayed. Even in its present state, the in vitro micronucleus is a reliable genotoxicity test. Compared with the chromosomal aberration test, it detects aneugens more reliably, it is faster and easier to perform, and it has more statistical power and the possibility of automation.

Minissi S, Lombi E. **Heavy metal content and mutagenic activity, evaluated by *Vicia faba* micronucleus test, of Tiber river sediments.** *Mutat Res* 1997;393(1-2):17-21.

BIOSIS COPYRIGHT: BIOL ABS. Tiber river sediment samples, collected in October 1995, were tested for mutagenicity by the micronucleus test in *Vicia faba* root tips. Four stations were studied within the urban area of Rome: (1) Castel Giubileo, at the entry of the urban area; (2) Ponte Tor di Quinto, immediately after the confluence of the tributary river Aniene; (3) Ponte Sublicio, in the middle of the city; and (4) Ponte della Magliana, immediately outside Rome, 20 km from the sea. Since no significant increase in micronucleus frequency was observed in any of the tested stations compared to control (while in previous campaigns mutagenic activity was observed in some of the same stations), it can be assumed an interesting recovery from mutagenic pollution in the 2 last years. The samples were analysed for pH value, nitrogen, organic matter and carbonate content, and the concentration of some potentially mutagenic heavy metal ions (Zn, Cd, Ni, V, Cu) was assessed. In all samples, a concentration of heavy metals higher than unpolluted areas was observed. However, the alkaline pH measured should keep them as non-bioavailable elements.

Misra RR, Smith GT, Waalkes MP. **Evaluation of the direct genotoxic potential of cadmium in four different rodent cell lines.** *Toxicology* 1998;126(2):103-14.

Cadmium is a toxic environmental contaminant that is carcinogenic in humans and laboratory animals. Although the mechanism underlying cadmium carcinogenesis has not yet been determined experimental evidence suggests that the stress-inducible, metal-binding proteins, metallothioneins, may mediate organ

specificity. In the present study, four different rodent cell lines (Chinese hamster ovary cells, rat L6 myoblast cells, rat Clone 9 liver cells, and rat TRL 1215 liver cells) were exposed to 0, 1, 5, 10, 50, or 100 microM CdCl₂ and monitored for evidence of direct DNA damage. A microfiltration assay was used to measure DNA strand breaks and a filter-binding assay was used to measure DNA-protein crosslinks, two lesions that have been associated with cadmium exposure and may mediate genotoxicity of the metal. Although variability in sensitivity to DNA damage was evident between the different cell lines, in all of the cell lines tested, increases in DNA damage were observed only at cadmium doses that completely arrested cell growth. In addition, in three of the four cell lines tested, induction of metallothionein had no substantial protective effect against cadmium-induced cytotoxicity or genotoxicity. While protection against cadmium-induced DNA strand breakage with metallothionein preinduction was observed in the TRL 1215 rat liver cells, metallothionein preinduction did not protect against cadmium-induced DNA-protein crosslinking in that cell line. Taken together, our results support the hypothesis that cadmium is not directly genotoxic.

Moller P, Wallin H, Grunnet N, Risom L, Knudsen LE. **DNA damage in isolated rat hepatocytes exposed to C.I. pigment orange 5 and C.I. pigment yellow 12 by the alkaline comet assay.** *Teratog Carcinog Mutagen* 1998;18(1):9-16.

BIOSIS COPYRIGHT: BIOL ABS. The induction of DNA damage by commonly used printing ink pigments, C.I. pigment orange 5 (C.I. 12075) and C.I. pigment yellow 12 (C.I. 21090), was investigated in freshly isolated rat hepatocytes with the comet assay. C.I. pigment yellow 12 is a 3,3'-dichlorobenzidine-based diarylide pigment, and C.I. pigment orange 5 is a naphthol-azo pigment. The pigments are virtually insoluble in aqueous solutions, and they have not been tested extensively for toxicological effects. C.I. pigment orange 5 increased the levels of DNA damage at 5 mug/ml ($P < 0.02$) and C.I. pigment yellow 12 at 20 mug/ml ($P < 0.002$). The effect of incubation time (20, 40, and 80 min) of the same concentrations of the pigments was tested. The levels of DNA damage were increased up to 80 min. Both pigments produced DNA damage that was in the same range as the food carcinogen 2-amino-3,8-dimethylimidazo(4,5f)quinoxaline. Our data indicate that both C.I. pigment orange 5 and C.I. pigment yellow 12 are genotoxic in hepatocytes with metabolizing capacities. However, further investigation of the metabolism and disposition are required for the evaluation of the safety of these pigments.

Moretti M, Villarini M, Scassellati-Sforzolini G, Santroni AM, Fedeli D, Falcioni G. **Extent of DNA damage in density-separated trout erythrocytes assessed by the 'comet' assay.** *Mutat Res* 1998;397(2):353-60.

The 'comet' assay is being increasingly employed for evaluating DNA damage in biological systems. Using this technique, we examined DNA damage in whole in density-separated trout erythrocytes. Results clearly show that all the three considered parameters (tail length, tail intensity and tail moment) increased with the density of the fractions, possibly reflecting different degrees of DNA damage. Probably, this behaviour is due to different periods of exposure of the density fractions to the hazard of active oxygen radicals; older cells have been exposed to oxidative stress for a longer time.

Morningstar ML, Kreutzer DA, Essigmann JM. **Synthesis of oligonucleotides containing two putatively mutagenic DNA lesions: 5-hydroxy-2'-deoxyuridine and 5-hydroxy-2'-deoxycytidine.**

Chem Res Toxicol 1997;10(12):1345-50.

Spontaneous oxidative DNA damage occurs as a consequence of aerobic metabolism, lipid peroxidation, immune responses, ionizing radiation, and some chemical oxidants. These processes yield a vast array of oxidized DNA bases and sugars. The existence of significant steady-state levels of oxidized DNA bases in the genome suggests that these lesions are not completely repaired on a biologically relevant time scale and thus may contribute to mutagenesis. In particular, studies have shown that the steady-state levels of 5-hydroxy-2'-deoxycytidine (dC5-OH) and its deamination product, 5-hydroxy-2'-deoxyuridine (dU5-OH), are similar to those found for 7,8-dihydro-8-oxoguanosine, a known highly mutagenic lesion formed by oxidation of guanosine. Structural and biological properties of dC5-OH and dU5-OH have been constrained by the lack of synthetic methodology for oligonucleotides containing these modified bases. A method is described here for the solid-phase synthesis of oligonucleotides containing dC5-OH and dU5-OH. Preparation of each of the required phosphoramidites involved the selective protection of the base 5-hydroxyl group over the deoxyribose 5'- and 3'-hydroxyl groups. The base composition and the incorporation of the adducts into synthetic heptanucleotides were confirmed after purification of the modified oligonucleotides by enzymatic digestion and HPLC analysis. Mass spectrometric analysis of the oligonucleotide products by electrospray MS and GC/MS further confirmed their composition. Most significantly, deamination of the dC5-OH oligomer to a putative dU5-OH product during solid-phase DNA synthesis or oligonucleotide deprotection was not detected by any analytical technique employed.

Nakagawa Y, Wakuri S, Sakamoto K, Tanaka N. **The photogenotoxicity of titanium dioxide particles.** Mutat Res 1997;394(1-3):125-32.

We employed a series of in vitro genotoxicity assays--a single cell gel (SCG) assay with mouse lymphoma L5178Y cells, a microbial mutation assay with Salmonella typhimurium, a mammalian cell mutation assay with L5178Y cells, and a chromosomal aberration assay with Chinese hamster CHL/IU cells--to evaluate the photogenotoxicity of titanium dioxide (TiO₂) particles. Without UV/visible light irradiation, TiO₂ particles exhibited no or weak genotoxicity. With irradiation, however, TiO₂ particles exhibited significant genotoxicity in the SCG and chromosomal aberration assays. Therefore, we concluded that TiO₂ particles are photogenotoxic.

Orkin SH, Zon LI. **Genetics of erythropoiesis: induced mutations in mice and zebrafish.** Annu Rev Genet 1997;31:33-60.

Production of red blood cells (erythropoiesis) in the vertebrate embryo is critical to its survival and subsequent development. As red cells are the first blood cells to appear in embryogenesis, their origin reflects commitment of mesoderm to an hematopoietic fate and provides an avenue by which to examine the development of the hematopoietic system, including the hematopoietic stem cell (HSC). We discuss the genetics of erythropoiesis as studied in two systems: the mouse and zebrafish (*Danio rerio*). In the mouse, targeted disruption has established several genes as essential at different stages of hematopoiesis or erythroid precursor cell maturation. In the zebrafish, numerous mutants displaying a wide range of phenotypes have been isolated, although the affected genes are unknown. In comparing mouse knockout and zebrafish mutant phenotypes, we propose a pathway for erythropoiesis that emphasizes the apparent similarity of the mutants and the complementary nature of investigation in the two species. We speculate that further genetic studies in mouse and zebrafish will identify the majority of essential genes and define a regulatory network for hematopoiesis in vertebrates.

Ozawa S. [**Genetic polymorphisms in xenobiotic metabolizing enzymes as a determinant of susceptibility to environmental mutagens and carcinogens in humans**]. *Yakugaku Zasshi* 1997;117 (10-11):895-909. (Jpn)

Xenobiotic metabolizing enzymes are known to play a role in the metabolic activation of environmental mutagens and carcinogens to exert their carcinogenic effects as well as detoxification by increasing their hydrophilicity. These enzymes include cytochrome P450s, glutathione S-transferases (GSTs), acetyltransferases (NATs) and sulfotransferases. Genetic polymorphisms in many of these enzymes, such as CYP1A1, CYP1A2, CYP2C9, CYP2D6, CYP2E1, NAT1, NAT2, GSTM1, GSTP1 and GSTT1, have been shown to occur, which result in the altered expression of enzymatic activities. This suggests that the genetic polymorphisms may affect the individual susceptibility to environmental carcinogens and thus play a role in human carcinogenesis. Recently, the mutations that confer those polymorphisms of xenobiotic metabolizing enzymes have been identified and genotyping methods for the genetic polymorphisms have been developed. Specific phenotypes and genotypes for CYP1A1, CYP2D6, CYP2E1, NAT1, NAT2, GSTM1 and GSTP1 have been associated with susceptibility to malignant diseases including lung, bladder and colon cancers, although the association was not confirmed in some studies. A number of factors such as degree of exposure to environmental carcinogens and the role of xenobiotic metabolizing enzymes in human carcinogenesis should carefully be evaluated in understanding genetic susceptibility.

Patel DJ, Mao B, Gu Z, Hingerty BE, Gorin A, Basu AK, Broyde S. **Nuclear magnetic resonance solution structures of covalent aromatic amine-DNA adducts and their mutagenic relevance**. *Chem Res Toxicol* 1998;11(5):391-407.

Perez-Alzola LP, Santos MJ. **In vitro genotoxic evaluation of conventional bleached and biobleached softwood pulp mill effluents**. *Mutat Res* 1997;395(2-3):107-12.

The effluents of pulp and paper mills contain about 300 different chemical compounds; many of them are mutagens and clastogens. Genotoxic studies have shown that chlorination stage liquors are significantly more genotoxic, in the Ames Salmonella assay, than the other process of lignin extraction, and that lyophilized effluents are genotoxic in cultured mammalian cells. Since these effluents from conventional bleaching stages are genotoxic, Chilean industries are interested in changing this process to a less toxic one, such as biobleaching using enzymes. In this study, we tested the in vitro genotoxicity of two types of effluents: an effluent obtained from a conventional radiata pine kraft-bleaching process (effluent D) and one derived from a biobleaching process with hemicellulase (effluent B). Both effluents were tested without any concentration or purification steps in the Ames Salmonella assay (TA100) and in the micronuclei (MN) and sister chromatid exchange (SCE) tests in CHO cells. The results showed that neither effluent induced base pair substitution mutations in the Ames Salmonella assay, and neither increased the micronucleus frequency in CHO cells. But, both increased the SCE frequencies in CHO cells, showing that this assay is more sensitive than the other ones, and that the two effluents contained chemical compounds in amounts enough to induce in vitro genotoxicity measured by the SCE induction.

Prival MJ, Zeiger E. **Chemicals mutagenic in Salmonella typhimurium strain TA1535 but not in TA100**. *Mutat Res* 1998;412(3):251-60.

The standard Salmonella mutagenicity test uses two strains of Salmonella typhimurium (TA1535 and TA100) containing the same base pair substitution mutation (hisG46). These strains differ only in that strain TA100 contains the plasmid pKM101, whose mucAB gene products enhance SOS mutagenesis. This makes strain TA100, in general, the more sensitive of the two for mutagen detection, raising the question as to whether or not to include strain TA1535 in the core battery of strains in routine testing. Out of 659 chemicals judged as mutagens in the S. typhimurium assay when subjected to the National Toxicology Program's screening protocol, 36 (5%) were evaluated as positive in strain TA1535 but not in strain TA100. Of these, 23 were judged as negative and 13 as equivocal in strain TA100, and 5 were positive or equivocal in at least one other strain (TA97 or TA98). In general, the data on these chemicals indicate that the absolute increases in revertants per plate induced in strain TA1535 were too small to have been judged as positive if similar increases occurred in strain TA100, which has a much higher spontaneous background. For three chemicals (acetaldehyde oxime, 6-mercaptopurine, and 1,3-butadiene) the absolute increases in revertants in strain TA1535 greatly exceeded those in strain TA100. Evaluation of the reproducibility of these findings and of the mechanisms and relevance of unique TA1535 positives should be useful when decisions are made as to whether this strain should be kept as a part of the core battery of strains in the S. typhimurium assay.

Privezentsev KV, Milonova IN, Bezlepkin VG. [Evaluation of CD and nitotoxicity and genotoxicity using algal toxicity test and SOS-Chromotest]. Usp Sovrem Biol 1995;115(6):759-64. (Rus)
BIOSIS COPYRIGHT: BIOL ABS. RRM LITERATURE REVIEW COSCINODISCUS-GRANII
ESCHERICHIA-COLI CADMIUM HEAVY METALS NICKEL GENOTOXICITY ALGAL
TOXICITY TEST SOS-CHROMOTEST TOXICOLOGY METHODOLOGY ECOTOXICOLOGY
HEAVY METAL TOXICITY ENVIRONMENTAL MONITORING TOXICITY TESTING METHOD.

Przybojewska B. **An evaluation of the DNA damaging effect of selected aniline derivatives using the alkaline single cell gel electrophoresis (comet) assay.** Mutat Res 1997;394(1-3):53-7.

The alkaline (pH > 13) single cell gel electrophoresis (SCGE) assay or comet assay was used to evaluate for DNA damage induced in bone marrow cells of B6C3F1 mice by four aniline derivatives 2,4-dimethylaniline (2,4-xylydine), 2,4,6-trimethylaniline (mesidine), 2-chloro-4-methylaniline and 4-chloro-N-methylaniline. The study revealed that two of the four compounds studied, i.e. 2,4-dimethylaniline and 2,4,6-trimethylaniline, increased the extent of DNA migration in bone marrow cells of mice. Two others. 2-chloro-4-methylaniline and 4-chloro-N-methylaniline, had no effect on the DNA of the cells in test conditions. The results of this study, in combination with those of other researchers. leads to the conclusion that 2,4-dimethylaniline and 2,4,6-trimethylaniline are genotoxic and potentially carcinogenic.

Ramos LA, Lipman R, Tomasz M, Basu AK. **The major mitomycin C-DNA monoadduct is cytotoxic but not mutagenic in Escherichia coli.** Chem Res Toxicol 1998;11(1):64-9.

To determine the mutagenic and genotoxic properties of the major guanine N2-adduct formed by the antitumor drug mitomycin C, we have synthesized a decanucleotide, d(TTACG[MC]TATCT), containing the adduct, which was inserted into a gapped bacteriophage M13 genome. Analysis of the constructed genome indicated that 41% ligation of the adducted 10-mer occurred on both sides of the gap, whereas the control 10-mer ligated with 34% efficiency. After transfection of the adducted single-

stranded M13 DNA into *Escherichia coli*, the adduct was found to be highly genotoxic. Viability of the adducted genome in a repair-competent strain was only 7%, which increased to 12% and 15% upon induction of SOS by irradiating the cells with 254-nm light at 20 and 50 J/m², respectively. Even lower viability of 2%, 4.6%, and 0.2% was observed in *uvrA*, *uvrB*, and *uvrC* strains, respectively, which increased up to 10-fold with SOS. An examination of the surviving phage populations revealed that the adduct was not detectably mutagenic. No mutants from the repair-proficient strain were detected after analysis of more than 2500 progeny phage. Only 0.2% of the survivors were mutants in the *uvrA* strain. It is uncertain, however, if they were induced by the adduct, since all the mutants showed untargeted mutations. We conclude that the major guanine N2-adduct formed by mitomycin C is cytotoxic but not appreciably mutagenic in *E. coli*.

Remington KM, Bennett SE, Harris CM, Harris TM, Bebenek K. **Highly mutagenic bypass synthesis by T7 RNA polymerase of site-specific benzo[a]pyrene diol epoxide-adducted template DNA.** *J Biol Chem* 1998;273(21):13170-6.

We have previously developed an *in vitro* system that allows quantitative evaluation of the fidelity of transcription during synthesis on a natural template in the presence of all four nucleotides. Here, we have employed this system using a TAA ochre codon reversion assay to examine the fidelity of transcription by T7 RNA polymerase past an adenine residue adducted at the N6-position with (-)-anti-trans- or (+)-anti-trans-benzo[a]pyrene diol epoxide (BPDE). T7 RNAP was capable of transcribing past either BPDE isomer to generate full-length run-off transcripts. The extent of bypass was found to be 32% for the (-)-anti-trans-isomer and 18% for the (+)-anti-trans-isomer. Transcription past both adducts was highly mutagenic. The reversion frequency of bypass synthesis of the (-)-anti-trans-isomer was elevated 11,000-fold and that of the (+)-anti-trans-isomer 6000-fold, relative to the reversion frequency of transcription on unadducted template. Adenine was misinserted preferentially, followed by guanine, opposite the adenine adducted with either BPDE isomer. Although base substitution errors were by far the most frequent mutation on the adducted template, three- and six-base deletions were also observed. These results suggest that transcriptional errors, particularly with regard to damage bypass, may contribute to the mutational burden of the cell.

Ribas G, Surralles J, Carbonell E, Xamena N, Creus A, Marcos R. **Genotoxic evaluation of the herbicide paraquat in cultured human lymphocytes.** *Teratog Carcinog Mutagen* 1997;17(6):339-47. The widely used herbicide paraquat was evaluated for genotoxicity in peripheral blood human lymphocyte cultures. Sister-chromatid exchanges (SCE), chromosome aberrations (CA), and micronuclei (MN) were scored as genetic endpoints. Paraquat was administered either alone or in combination with an external source of metabolic activation. Our data indicate that paraquat induced slight but significant increases in the frequency of SCE. This genotoxic effect was not modified by cotreatments with S9 fraction from rat liver. However, paraquat did not increase the frequency of CA and MN, indicating that this bipyridylium compound is not effective in these assays, which would mean a general lack of effectiveness of the herbicide to induce clastogenic damage. In addition, cotreatments with the S9 fraction, did not modify the genotoxic ability detected in the SCE assay.

Rodriguez-Arnaiz R. **Genotoxic activation of hydrazine, two dialkylhydrazines, thiourea and ethylene thiourea in the somatic w/w + assay of *Drosophila melanogaster*.** *Mutat Res* 1997;395(2-

3):229-42.

Genotoxic activation of hydrazine (HZ), two symmetrical dialkylhydrazines, namely, 1,2-dimethylhydrazine and 1,2-diethylhydrazine (SDMH and SDEH), thiourea (TU) and ethylene thiourea (ETU) has been evaluated by means of the w/w + somatic assay of *Drosophila*. Both low bioactivation insecticide-susceptible (IS) and high biotransformation insecticide-resistant (IR) strains were used. The combined application of insecticide-susceptible and insecticide-resistant strains should, in principle, detect somatic cell recombinagens in the *Drosophila melanogaster* in vivo w/w + assay. The IS strain was more susceptible to toxicity induced by the test chemicals than the IR stocks. Its performance in the biotransformation of the chemicals tested was rather poor. TU was inactive in all strains. With the active compounds, spot frequencies increased approximately linearly with dose for each spot type. SDEH gave a strong positive result in all three female genotypes exposed. HZ, ETU and SDMH were overall weakly positive in the IR strain Haag-79 (HG-R). Interestingly, ETU was clearly positive in the IR Hikone-R (HK-R) strain. A comparison of the recombinagenic potencies between the active and the weakly positive compounds, and among strains, showed pronounced genotype-dependent differences between the low and the high bioactivation strains. The ability of *Drosophila* to express several procarcinogens in relation to insecticide-resistance after activation catalyzed by CYP450 enzymes is discussed.

Rozinova E, Khalil M, Bonin AM. **MOCA and some proposed substitutes (Cyanacure, Conacure, Polacure 740M and Ethacure 300) as two-stage skin carcinogens in HRA/Skh hairless mice.** *Mutat Res* 1998;398(1-2):111-21.

4,4'-Methylenebis(2-chlororaniline) (MOCA) is a suspect human carcinogen that has wide use as an industrial compound. Occupationally, exposure may occur through inhalation and ingestion, but skin absorption is the main route by which this compound gains entry into the body. Because of the justified concern about the continued use of MOCA, a number of substitutes have been proposed, including 1,2-bis(2-aminophenylthio)ethane (Cyanacure), Conacure, trimethylene glycol di-p-aminobenzoate (Polacure 740M) and 3,5-dimethylthio-2,4-toluenediamine/3,5-dimethylthio-2,6-toluenediamine (Ethacure 300). There is very little information available about these substances, but they share the property of belonging to the same class (aromatic amines) as MOCA. Furthermore, at least two (Ethacure 300 and Cyanacure) are mutagenic in *Salmonella*. This study was undertaken to investigate if MOCA and substitutes, Polacure 740M, Ethacure 300, Cyanacure and Conacure have the potential to cause papillomas in a two stage initiation/promotion protocol in HRA/Skh hairless mice. When a maximum dose of 100 mg of substance was applied to the dorsal skin of these mice, Ethacure 300 and Cyanacure were markedly toxic. All of the compounds had little or no effect on skin tumor initiating activity following 12-O-tetradecanoylphorbol-13-acetate (TPA) promotion. One experiment with MOCA suggested that, at lower and less toxic dose, this substance may have promotional activity. Therefore, caution should still be exercised when using these compounds and it cannot be excluded that they may be active in other strains of mice or other laboratory animal species.

Saranko CJ, Recio L. **The butadiene metabolite, 1,2:3,4-diepoxybutane, induces micronuclei but is only weakly mutagenic at lacI in the Big Blue Rat2 lacI transgenic cell line.** *Environ Mol Mutagen* 1998;31(1):32-40.

1,3-Butadiene (BD) is a genotoxic carcinogen that is bioactivated to at least two mutagenic metabolites,

1,2-epoxybutene (EB) and 1,2:3,4-diepoxybutane (DEB). We investigated the mutagenicity and induction of micronuclei by DEB in vitro in Rat2 lambda/lacI transgenic fibroblasts (Big Blue Rat2 cells, Stratagene, LaJolla, CA). Assays for mutagenicity and micronuclei induction were carried out at concentrations of 0, 2, 5, or 10 microM DEB for 24 hours. Exposure of cells to these concentrations of DEB resulted in approximately 100, 50, and 10% survival, respectively, compared with media controls. In independent replicate experiments, no statistically significant increase in lacI mutant frequency was observed in Rat2 cells at any of the DEB exposure concentrations when compared to media or solvent controls. However, regression analyses indicated a trend toward increasing mutant frequency with increasing DEB exposure concentration. Experiments to examine the induction of micronuclei by DEB revealed a concentration-dependent increase in micronuclei in Rat2 cells following exposure to DEB. These results indicate that DEB induces micronuclei in the absence of detectable gene mutation at lacI in Big Blue Rat2 cells. The induction of micronuclei but only weak mutagenicity at the lacI transgene is likely due to the poor recovery of deletions using this lambda shuttle vector system, demonstrating the need to investigate multiple endpoints of genotoxicity when considering the mutational activity of a compound.

Sardas S, Aygun N, Karakaya AE. **Genotoxicity studies on professional hair colorists exposed to oxidation hair dyes.** *Mutat Res* 1997;394(1-3):153-61.

The cytogenic repercussions of occupational exposure to oxidation hair dyes were assessed by using three assays in professional hair colorists. The assays were sister chromatid exchanges (SCE) in circulating lymphocytes to evaluate the interchange of DNA replication products at apparently homologous chromosomal loci, single cell gel electrophoretic (SCGE) assay to detect the presence of DNA strand breaks/alkali-labile damage, and the Ames assay using *Salmonella typhimurium* strain TA98 to detect the urine mutagenicity. The ability of these assays to detect genetic damage caused by oxidation hair dyes in man compared with closely matched controls produced the following findings. (i) The SCE assay could not detect the mutagenic effect in lymphocytes of exposed subjects from whom complete data were obtained. However, subjects (controls and exposed) with a history of smoking had slightly increased SCEs than the non-smokers in both groups. (ii) The extent of DNA migration (SCGE assay) did not distinguish between the samples in either the exposed or control subjects. Like the SCE results, the exposed and control smoker subjects showed a greater proportion of damaged lymphocytes with apparent migration of DNA. (iii) No clear differences in the mutagenic activity of the urine samples were observed between the exposed and control subjects. But, pooling exposed and controls together, a positive and significant variation in the urinary mutagenic effect was observed with the number of cigarettes smoked per day.

Sasaki YF, Saga A, Akasaka M, Nishidate E, Watanabe-Akanuma M, Ohta T, Matsusaka N, Tsuda S. **In vivo genotoxicity of heterocyclic amines detected by a modified alkaline single cell gel electrophoresis assay in a multiple organ study in the mouse.** *Mutat Res* 1997;395(1):57-73.

We used a modification of the alkaline single cell gel electrophoresis (SCG) (Comet) assay to test the in vivo genotoxicity of 6 heterocyclic amines, Trp-P-1 (25 mg/kg), Trp-P-2 (13 mg/kg), IQ (13 mg/kg), MeIQ (13 mg/kg), MeIQx (13 mg/kg) and PhIP (40 mg/kg), in mouse liver, lung, kidney, brain, spleen, bone marrow and stomach mucosa. Mice were sacrificed 1, 3, and 24 h after intraperitoneal injection. Trp-P-2, IQ, MeIQ, and MeIQx yielded statistically significant DNA damage in the stomach, liver,

kidney, lung and brain; Trp-P-1 in the stomach, liver and lung; and PhIP in the liver, kidney and brain. None of the heterocyclic amines induced DNA damage in the spleen and bone marrow. Our results suggest that the alkaline SCG assay applied to multiple organs is a good way to detect organ-specific genotoxicity of heterocyclic amines in mammals.

Sasaki YF, Saga A, Akasaka M, Yoshida K, Nishidate E, Su YQ, Matsusaka N, Tsuda S. **In vivo genotoxicity of ortho-phenylphenol, biphenyl, and thiabendazole detected in multiple mouse organs by the alkaline single cell gel electrophoresis assay.** *Mutat Res* 1997;395(2-3):189-98.

In Japan, ortho-phenylphenol (OPP), biphenyl (BP), and thiabendazole (2-(4'-thiazolyl)benzimidazole, TBZ) are commonly used as a postharvest treatment to preserve imported citrus fruits during transport and storage. We used a modification of the alkaline single cell gel electrophoresis (SCG) (Comet) assay to test the in vivo genotoxicity of those agents in mouse stomach, liver, kidney, bladder, lung, brain, and bone marrow. CD-1 male mice were sacrificed 3, 8, and 24 h after oral administration of the test compounds. OPP (2000 mg/kg) induced DNA damage in the stomach, liver, kidney, bladder, and lung, BP (2000 mg/kg) and TBZ (200 mg/kg) induced DNA damage in all the organs studied. For OPP, increased DNA damage peaked at 3-8 h and tended to decrease at 24 h. For BP, on the contrary, increased DNA migration peaked at 24 h. That delay may have been due to the fact that OPP is metabolized by cytochrome 450 and prostaglandin H synthase to phenylbenzoquinone (PBQ), a DNA binding metabolite, and BP is metabolized to PBQ via OPP and m-phenylphenol. The positive response to TBZ, an aneugen, supports the in vivo DNA-damaging action of TBZ.

Schimmer O, Eschelbach H, Breiting DK, Grutzner T, Wick H. **Organogermanium compounds as inhibitors of the activity of direct acting mutagens in Salmonella typhimurium.**

Arzneimittelforschung 1997;47(12):1398-402.

The organogermanium compounds bis(D,L-lactato)germanium(IV), bis(L-lactato)germanium(IV), bis(thiolactato)germanium(IV) and bis(thioglycolato)germanium(IV) were tested for their antimutagenic activity in *Salmonella typhimurium* strains TA98 and TA100. Each compound showed moderate activity against the mutagenic effect of nitroaromatic compounds and weak effects against the mutagenic activity of ethylmethane sulfonate. No inhibition of mutagenicity was observed against the indirect acting promutagens benzo(a)pyrene and 2-aminoanthracene. The compounds differed only quantitatively in their antimutagenicity spectrum. It is concluded from these results that an intracellular mechanism is involved in the inhibition of ethylmethane sulfonate-induced mutagenicity. The effect is probably produced, at least partially, at the level of DNA repair. Frameshift mutations seem to be prevented with higher efficiency than base pair substitutions.

Schmezer P, Eckert C, Liegibel UM, Klein RG, Bartsch H. **Use of transgenic mutational test systems in risk assessment of carcinogens.** *Arch Toxicol Suppl* 1998;20:321-30.

Two transgenic in vivo mutation assays are described which are based on LacZ (Muta Mouse) and LacI (Big Blue) shuttle vector systems. Their utility has already been explored by a number of investigators including our laboratory. The evaluation of data derived from these assays confirm that they offer a practical method for studying mutagenic activity and mechanism in a wide range of tissues including those of the respiratory and gastrointestinal tract. Therefore, these transgenic mutation assays are valuable tools to assess the organotropic effects of genotoxic carcinogens.

Shima N, Xiao LZ, Sakuramoto F, Ichikawa S. **Young inflorescence-bearing shoots with roots of Tradescantia clone BNL 4430 cultivated in nutrient solution circulating systems: an alternative to potted plants and cuttings for mutagenicity tests.** *Mutat Res* 1997;395(2-3):199-208.

The use of young inflorescence-bearing shoots with roots of Tradescantia clone BNL 4430 cultivated in a nutrient solution circulating (NSC) growth chamber was tested and developed as an alternative method for using Tradescantia plants in mutagenicity testings. The NSC growth chamber was designed for our requirements, based on trial cultivations of the shoots with roots in its smaller-sized prototype. The nutrient solution used was a 1/2500 Hyponex solution. The characteristics of this clone, i.e., many new shoots constantly emerging from the basal nodes one after another and its short height favorable for early flowering, made it possible to prepare many young inflorescence-bearing shoots with roots at one time. A simplified NSC cultivation system could also be developed at a lower cost, and by using it together with the NSC growth chamber, recycling of untreated materials was established for supplying steadily enough amounts of young inflorescence-bearing shoots with roots for mutagenicity testings. Compared with traditional methods of using potted plants or cuttings, the new method exhibited more stable flower production, better stamen-hair growth and a significantly lower spontaneous (background) mutation frequency, and could produce more inflorescences per space. The use of such young inflorescence-bearing shoots with roots was therefore judged to be satisfactory to serve as a new mutagenicity test system alternating with potted plants and cuttings.

Speit G, Dennog C, Lampl L. **Biological significance of DNA damage induced by hyperbaric oxygen.** *Mutagenesis* 1998;13(1):85-7.

Hyperbaric oxygen (HBO) treatment as used therapeutically has been shown to induce DNA damage in the alkaline comet assay with leukocytes from test subjects. Using formamidopyrimidine-DNA glycosylase, a DNA repair enzyme which specifically nicks DNA at sites of 8-oxoguanines and formamidopyrimidines, we have detected enhanced DNA migration, indicating significant oxidative base damage, after HBO treatment. Increased DNA damage was seen immediately at the end of treatment, while 24 h later no effect was found. We now show that HBO-induced DNA strand breaks and oxidative base modifications are rapidly repaired, leading to a reduction in induced DNA effects of > 50% during the first hour. A similar decrease was found in blood taken immediately after exposure and post-incubated for 2 h at 37 degrees C in vitro and in blood taken and analysed 2 h after exposure, suggesting similar repair activities in vitro and in vivo. When the same blood samples showing increased DNA damage after HBO in the comet assay were analysed in the micronucleus test, no indications of induced chromosomal breakage in cultivated leukocytes could be obtained. The results suggest that the HBO-induced DNA effects observed with the comet assay are efficiently repaired and are not manifested as detectable chromosome damage.

Steinkellner H, Mun-Sik K, Helma C, Ecker S, Ma TH, Horak O, Kundi M, Knasmuller S. **Genotoxic effects of heavy metals: comparative investigation with plant bioassays.** *Environ Mol Mutagen* 1998;31(2):183-91.

The potential use of micronucleus assays in plants for the detection of genotoxic effects of heavy-metal ions was investigated. Three different plant systems were comparatively investigated in micronucleus tests with Tradescantia pollen mother cells (Trad MCN) and micronucleus tests with meristematic root

tip cells of *Allium cepa* and *Vicia faba* (*Allium/ Vicia* MCN). As³⁺, Pb²⁺, Cd²⁺, Zn²⁺ caused a dose-dependent increase of MCN frequencies in all three test systems. Cu²⁺ gave consistently negative responses in all three tests; Zn²⁺ caused only a moderate, statistically not significant increase of MCN frequencies in *Vicia*. The ranking of genotoxic potencies in all three tests was in the descending order: As³⁺ > Pb²⁺ > Cd²⁺ > Zn²⁺ Cu²⁺. In experiments with *Tradescantia*, induction of MCN was observed in a concentration range between 1 and 10 mM, whereas in tests with root tip cells, higher concentrations (10-1,000 mM) were required to show significant effects. Further increase of the exposure levels caused toxic effects (reduction of root growth), cell division delays, and a decrease of MCN frequencies. Comparisons by linear regression analyses indicated that the sensitivity of the three bioassays for heavy metals decreases in the order: Trad MCN > *Vicia* root MCN > *Allium* root MCN. In further experimental series, a soil sample which contained high concentrations of the five metals and a control soil were investigated. Aqueous soil extracts induced only weak effects in Trad MCN tests and no effects in the root tip assays, whereas cultivation of the plants in the soils resulted in a pronounced induction of MCN in the *Tradescantia* system and moderate effects in *Vicia* and *Allium*. In conclusion, the results of the study indicate that the Trad MCN assay detects the genotoxic effects of heavy metals and can be used for biomonitoring metal-contaminated soils.

Suri A, Deboer J, Kusser W, Glickman BW. **A 3 milliTesla 60 Hz magnetic field is neither mutagenic nor co-mutagenic in the presence of menadione and MNU in a transgenic rat cell line.** *Mutat Res* 1996 Nov 11;372(1):23-31.

The mechanisms by which an electromagnetic field (EMF) influences biological material are poorly understood. One potentially important model suggests that a magnetic field can stabilize free radicals in such a way as to permit their dispersement rather than their return to the ground state (Okazaki et al., 1988; Scaiano, 1995). We have tested this hypothesis by examining mutagenesis in the *E. coli* lacI gene target carried in the Big Blue rat embryo fibroblast cell line, R2 lambda LIZ. Mutant frequencies were determined in cells exposed to a magnetic field, cells pretreated with the mutagens N-methylnitrosourea (MNU) or 2-methyl-1,4-naphthoquinone (menadione), prior to being held in a 60 Hz 3 milliTesla (mT) magnetic field and cells concurrently exposed to the mutagens and the magnetic field. Menadione was selected because its mutagenic mechanism involves the formation of free radicals, while MNU is an alkylating agent not thought to act through radical formation. According to the radical stabilization hypothesis the application of a magnetic field to menadione treated cells would accentuate the mutagenic effects. Our results failed to indicate that the magnetic field affects mutagenesis by the oxygen-radical mediated mutagen, menadione.

Suzuki A, Kushida H, Iwata H, Watanabe M, Nohmi T, Fujita K, Gonzalez FJ, Kamataki T. **Establishment of a Salmonella tester strain highly sensitive to mutagenic heterocyclic amines.** *Cancer Res* 1998;58(9):1833-8.

Heterocyclic amines (HCAs) that are present in cooked foods require metabolic activation to exert their genotoxicity. They undergo activation via N-hydroxylation by cytochrome P450 1A2 (CYP1A2), followed by O-esterification by O-acetyltransferase (OAT). To develop a *Salmonella* tester strain that is highly sensitive to mutagenic HCAs, we introduced a coexpression plasmid (p1A2OR) carrying human CYP1A2 and NADPH-CYP reductase cDNAs and an expression plasmid (pOAT) carrying *Salmonella* OAT to *Salmonella typhimurium* TA1538 to yield a TA1538/ARO strain. The TA1538/ARO strain was

proven to express the enzymes, as indicated by high activities of 7-ethoxyresorufin O-deethylase and isoniazid N-acetylase. The TA1538/ARO strain exhibited very high sensitivity to mutagenic HCAs 2-amino-3,4-dimethylimidazo[4,5-f]quinoline, 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline and a somewhat higher sensitivity to 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine compared with the parent Ames tester strain TA1538. The minimum concentrations of 2-amino-3,4-dimethylimidazo[4,5-f]quinoline, IQ, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline, and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine giving positive results were defined by evidence that the number of colonies increased in a dose-dependent manner and reached a number two times higher than that obtained by vehicle alone as a control in the TA1538/ARO strain at concentrations of 0.3, 3, 30, and 1000 pM, respectively. When the membrane and cytosol fractions prepared from TA1538/ARO were added to a mixture containing the parental TA1538, the sensitivity of TA1538 to IQ was much lower than that seen with TA1538/ARO. These results indicate that the intracellular expression of drug-metabolizing enzymes makes the established strain of Salmonella highly sensitive to mutagenic HCAs.

Tice RR, Furedi-Machacek M, Satterfield D, Udumudi A, Vasquez M, Dunnick JK. **Measurement of micronucleated erythrocytes and DNA damage during chronic ingestion of phenolphthalein in transgenic female mice heterozygous for the p53 gene.** *Environ Mol Mutagen* 1998;31(2):113-24. Phenolphthalein, a common ingredient in nonprescription laxatives and a multisex, multispecies rodent carcinogen, was evaluated under chronic exposure conditions for genotoxicity in transgenic female mice heterozygous for the p53 gene (heterozygous TSG-p53 mice). Phenolphthalein was administered in the diet at 200, 375, 750, 3,000, and 12,000 ppm (corresponding to a time-weighted average of 37, 71, 146, 569, and 2,074 mg/kg/day, respectively) for 6 months (183 days). On days 39, 92, 137, and 183 of treatment, peripheral blood samples were collected and evaluated for the frequency of micronucleated polychromatic and normochromatic erythrocytes (MN-PCE and MN-NCE, respectively), the percentage of PCE (%PCE) among total erythrocytes, and the extent of DNA damage (single strand breaks, alkali labile sites, DNA crosslinking) in leukocytes. In addition, the extent of DNA damage was evaluated in liver parenchymal cells sampled from mice at the end of the 6-month treatment period. DNA damage was evaluated using the alkaline (pH > 13) Single Cell Gel (SCG) assay. In addition, using a modified SCG technique, the frequencies of leukocytes and liver parenchymal cells with extremely low molecular weight DNA (indicative of apoptosis and/or necrosis) were determined. At each sample time, phenolphthalein induced a highly significant, dose-dependent increase in the frequency of MN-PCE and MN-NCE and in %PCE. Maximal induction of MN-PCE and %PCE decreased with increasing treatment duration, most likely due to a treatment duration-dependent decrease in the relative amount of ingested phenolphthalein. A comparative analysis of the kinetochore status of MN in erythrocytes sampled from control mice and mice ingesting phenolphthalein at 12,000 ppm for 183 days indicates that the induced MN resulted predominantly but not exclusively from numerical chromosomal damage. The analysis for increased levels of DNA damage in blood leukocytes was inconclusive, with a small but statistically significant increase in DNA migration on days 39 and 137 but not on days 92 and 183. The extent of DNA migration in liver parenchymal cells sampled from mice at the end of treatment was not altered significantly. The frequencies of apoptotic and/or necrotic leukocytes and liver parenchymal cells were not increased among mice ingesting phenolphthalein. The lowest effective dose at which a significant genotoxic response (i.e., the induction of MN-NCE) was detected was 200 ppm, the lowest

dose tested in this study. This dose in mice is comparable to doses (on a mg/m² basis) experienced by humans.

Trinh S, Reysset G. Mutagenic action of 5-nitroimidazoles: in vivo induction of GC-->CG transversion in two Bacteroides fragilis reporter genes. Mutat Res 1998;398(1-2):55-65.

The in vivo mutagenic potential of two 5-nitroimidazoles, metronidazole and dimetridazole, was evaluated in *Bacteroides fragilis*, a strictly anaerobic bacterium. Two antibiotic resistance genes, tetA(Q) 3 and nimA, were used as DNA targets. The forward and back mutations were identified by nucleotide sequence analysis. Both drugs induced GC-->CG transversion exclusively. The results suggest that the reactive molecules generated during the intracellular reduction of the 5-nitroimidazoles are responsible for both base pair substitutions and DNA strand breaks, although the mechanisms and targets may be different.

Tuppurainen K. A plausible mechanism for the mutagenic activity (Salmonella typhimurium TA100) of MX compounds: a formation of CG-CG(+)-CG radical cation by one-electron reduction. SAR QSAR Environ Res 1997;7(1-4):281-6.

Combining our previous QSAR work with recent high-level quantum mechanical calculations, a plausible mechanism for the mutagenic activity of halogenated furanones (so called MX compounds) in *Salmonella typhimurium* TA100 tester strain is proposed. The mechanism involves one-electron reduction as a key step and it seems reasonable to suggest that the mutagenicity of these direct-acting compounds may be a purely thermodynamic phenomenon, rather than the result of site-specific binding or adduct formation. Overall, the proposed model is consistent with the most experimental findings.

Ukawa-Ishikawa S, Sawada A, Kasuya K, Mochizuki M. Mutagenicity of isomeric alkanediazotates, precursors for ultimate alkylating species of carcinogenic N-nitroso compounds. Mutat Res 1998;412(1):99-107.

Alkanediazohydroxides are common key intermediates in carcinogenesis and mutagenesis of N-nitroso compounds, which are widely found in human environment. Mutagenicity of (E)- and (Z)-potassium alkanediazotates, as precursors of corresponding alkanediazohydroxides were evaluated to investigate the effect of geometric isomerism and also the effect of alkyl groups on their biological activity. Mutagenicity of N-nitroso-N-alkylureas which spontaneously produce alkanediazohydroxides after non-enzymatic hydrolysis were also tested in comparison to that of the corresponding diazotates and other activated chemical species of N-nitrosamines. When the mutagenicity was assayed in three microbial strains, *Salmonella typhimurium* TA1535, and *Escherichia coli* WP2 and WP2 uvrA, the order of mutagenic potency of the compounds with the same alkyl group was as follows; (E)-diazotates > (Z)-diazotates > nitrosoureas. The effect of alkyl groups on the mutagenic potency was different in *Salmonella* strain and in *E. coli* strains, and this result could be explained by the efficiency of O6-alkylguanine-DNA alkyltransferase. In each bacterial strain, this effect of alkyl groups was similar in mutagenicity induced by (E)- and (Z)-diazotates, N-nitroso-N-alkylureas and other activated N-nitrosodialkylamines such as alpha-hydroxy nitrosamines. The geometrical isomerism affected the mutagenicity of (E)- and (Z)-potassium alkanediazotates, and the result suggested that alkanediazohydroxides react through diazonium ions in a cage rather than through free alkyldiazonium ions which have no geometrical isomerism. Our results confirmed that (E)-potassium alkanediazotates,

(Z)-potassium alkanediazotates and N-nitroso-N-alkylureas all decomposed through diazohydroxides, and that alkanediazohydroxides are the active alkylating species of N-nitroso compounds, and also that the geometrical isomerism is important for carcinogenic N-nitroso compounds to show their biological activity.

Venturi M, Hambly RJ, Glinghammar B, Rafter JJ, Rowland IR. **Genotoxic activity in human faecal water and the role of bile acids: a study using the alkaline comet assay.** *Carcinogenesis* 1997;18(12):2353-9.

Human faecal waters from 35 healthy non-smoking volunteers (23 from England and 12 from Sweden) consuming their habitual diet were screened for genotoxicity by the single-cell gel electrophoresis (comet) assay using a human colon adenocarcinoma cell line (CACO-2) as the target. Hydrogen peroxide induced DNA damage was categorized as low, intermediate or high for tail moments greater than 5, 17 and 32, respectively: 11 samples were highly genotoxic, four were intermediate, one was low and 19 showed no activity. Endonuclease III treatment significantly increased DNA damage for all except the non-genotoxic faecal waters, suggesting that faecal water genotoxicity may be due, at least in part, to oxidative damage. Faecal water cytotoxicity has previously been attributed to the bile and fatty acid content. In the comet assay no DNA damage was induced by deoxycholate or lithocholate at normal physiological concentrations, suggesting that the genotoxicity of faecal water was due to other substances. Both bile acids induced DNA damage above 300 microM, levels often found in patients with colonic polyps and there was a significant increase in genotoxicity after endonuclease III treatment indicative of oxidative DNA damage.

Wagner ED, Rayburn AL, Anderson D, Plewa MJ. **Calibration of the single cell gel electrophoresis assay, flow cytometry analysis and forward mutation in Chinese hamster ovary cells.** *Mutagenesis* 1998;13(1):81-4.

The induction kinetics of genetic damage were measured in one clone of a mammalian cell line (CHO AS52) with three genotoxicity assays, the single cell gel electrophoresis (Comet) assay, laser beam flow cytometry and forward mutation. The first two assays allow for the rapid analysis of genotoxic damage in individual nuclei. The alkaline Comet assay detects DNA strand breaks, alkali-labile sites and incomplete excision repair sites. Flow cytometry measures chromosome damage that results in an unequal distribution of nuclear DNA in daughter cells. We calibrated these assays to compare acute DNA damage and longer term clastogenicity with forward mutation at the gpt locus using ethyl methanesulfonate (EMS). The EMS treatments were conducted in F12 medium for 2 h. AS52 cells carry a single functional gpt gene which provides for quantitation of gpt mutants by selecting for 6-thioguanine resistance. EMS induced a concentration-dependent response with median Comet tail moment values of 1.06 microns for the negative control and 64.6 microns with 20 mM. The coefficient of variation (CV) of the negative-control with flow cytometry was 2.33; the CV value increased to 4.87 in cells treated with 20 mM EMS, EMS (8 mM) induced a mutant frequency of 779.8×10^{-6} at a relative survival of 64.4%. Genetic response factors were calculated and the data demonstrate that the induction kinetics of genetic damage as measured by the Comet assay (15.6) and flow cytometry (14.2) were more closely related than that determined for mutation induction (7.9). These three assays measure a wide spectrum of genetic events at the level of DNA, the gene and the chromosome and demonstrate the usefulness of the Comet assay and flow cytometry as two relatively rapid procedures to detect

genotoxic damage in mammalian cells.

Watanabe T, Takashima M, Kasai T, Hirayama T. **Comparison of the mutational specificity induced by environmental genotoxin nitrated polycyclic aromatic hydrocarbons in Salmonella typhimurium his genes.** *Mutat Res* 1997;394(1-3):103-12.

Mutagenicity of 15 nitrated polycyclic aromatic hydrocarbons (nitro PAHs), which were detected in ambient air particles and/or combustion source emissions, were examined using a set of six *Salmonella typhimurium* tester strains (TA7001 to TA7006), and the mutational specificity was characterized by the comparison of the mutagenic potencies of nitro-PAHs in the tester strains. Each strain carries a unique missense mutation in the histidine operon and is reverted by only one specific base-substitution out of six possible changes. All nitro-PAHs tested were mutagenic in multiple strains, and were classified into four categories based on the strains predominantly reverted. 1-Nitropyrene (1-NPy), 2,7-dinitrofluoren-9-one and 1,3-, 1,6- and 1,8-dinitropyrene isomers exerted the highest mutagenicity in strain TA7005 (C.G-->A.T transversion) followed by strain TA7006 (C.G-->G.C transversion). 2- And 3-nitrofluoren-9-one isomers, 2-NPy and 2,7-dinitrophenanthrene were also markedly mutagenic in strain TA7005 but not in strain TA7006. For 2-, 3- and 9-nitrophenanthrene isomers, 2-nitrofluoranthene (2-NFT) and 4-NPy, TA7004 (G.C-->A.T transition) was the most responsive strain. 3-NFT was unique, showing the highest mutagenicity in strain TA7002 (T.A-->A.T transversion). All nitro-PAHs tested induced C.G-->A.T transversion, which is observed as the most frequent base-substitution mutation of p53 tumor suppressor gene in human lung cancer.

HEPATIC AND RENAL TOXICITY

Adinehzadeh M, Reo NV. **Effects of peroxisome proliferators on rat liver phospholipids: sphingomyelin degradation may be involved in hepatotoxic mechanism of perfluorodecanoic acid.** *Chem Res Toxicol* 1998;11(5):428-40.

Beckwith-Hall BM, Nicholson JK, Nicholls AW, Foxall P J, Lindon JC, Connor SC, Abdi M, Connelly J, Holmes E. **Nuclear magnetic resonance spectroscopic and principal components analysis investigations into biochemical effects of three model hepatotoxins.** *Chem Res Toxicol* 1998;11(4):260-72.

BIOSIS COPYRIGHT: BIOL ABS. ¹H NMR spectroscopy of urine combined with pattern recognition (PR) methods of data analysis has been used to investigate the time-related biochemical changes induced in Sprague-Dawley rats by three model hepatotoxins: alpha-naphthyl isothiocyanate (ANIT), D-(+)-galactosamine (GalN), and butylated hydroxytoluene (BHT). The development of hepatic lesions was monitored by conventional plasma analysis and liver histopathology. Urine was collected continuously postdosing up to 144 h and analyzed by 600-MHz ¹H NMR spectroscopy. NMR spectra of the urine samples showed a number of time-dependent perturbations of endogenous metabolite levels that were characteristic for each hepatotoxin. Biochemical changes common to all three hepatotoxins included a reduction in the urinary excretion of citrate and 2-oxoglutarate and an increased excretion of taurine and creatine. Increased urinary excretion of betaine, urocanic acid, tyrosine, threonine, and glutamate was characteristic of GalN toxicity. Both GalN and ANIT caused increased urinary excretion of bile acids, while glycosuria was evident in BHT- and ANIT-treated rats. Data reduction of the NMR spectra into

256 integrated regions was used to further analyze the data. Mean values of each integrated region were analyzed by principal components analysis (PCA). Each toxin gave a unique time-related metabolic trajectory that could be visualized in two-dimensional PCA maps and in which the maximum distance from the control point corresponded to the time of greatest cellular injury (confirmed by conventional toxicological tests). Thereafter, the metabolic trajectories changed direction and moved back toward the control region of the PR map during the postdose recovery phase. The combination of urinary metabolites which were significantly altered at various time points allowed for differentiation between biliary and parenchymal injury. This NMRPR approach to the noninvasive detection of liver lesions will be of value in furthering the understanding of hepatotoxic mechanisms and assisting in the discovery of novel biomarkers of hepatotoxicity.

Furitsu H, Ogawara K, Fujita T, Yamashita F, Hashida M, et al. **Pharmacokinetic analysis of scavenger receptor mediated uptake of anionized proteins in the isolated perfused rat liver.** *Int J Pharm* 1997 May 12;151:15-26.

IPA COPYRIGHT: ASHP The hepatic uptake of various anionized proteins by scavenger receptors in isolated perfused rat liver was studied. The molecular weight and total number of anionic charges per protein molecule were important for the hepatic uptake of anionized proteins. Hepatic uptake of succinylated bovine albumin proceeded via receptor mediated endocytosis. The internalization rate constant for succinylated bovine albumin was calculated to be 0.27 min⁻¹ in liver perfusion studies using the acid wash method. The outflow patterns of this protein in various inflow concentrations were fitted to a physiological one organ pharmacokinetic model. The obtained pharmacokinetic parameters for succinylated bovine albumin clearly characterized the difference in hepatic uptake mechanisms compared with lactosylated and cationized bovine albumin.

Hayashi F, Motoki Y, Tamura H, Watanabe T, Ogura T, Esumi H, Suga T. **Induction of hepatic poly (ADP-ribose) polymerase by peroxisome proliferators, non-genotoxic hepatocarcinogens.** *Cancer Lett* 1998;127(1-2):1-7.

Two peroxisome proliferators, [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio] acetic acid (Wy-14,643) or di(2-ethylhexyl) phthalate (DEHP), were given orally to male F-344 rats for up to 78 or 97 weeks. At 1 week, the activity of poly(ADP-ribose) polymerase (pADPRP) was increased 2- and 1.8-fold in the liver of rats treated with Wy-14,643 and DEHP, respectively. The induction of the activity was maintained at 2.5- or 2-fold for up to 52 weeks. The immunoblot and Northern blot analyses revealed that the induction of pADPRP activity would be responsible for the increase in the amount of mRNA. In addition, in the liver tumor induced by Wy-14,643 and DEHP, the pADPRP mRNA level increased 3.6- or 3.7-fold. The magnitude of the increase in the mRNA level was higher than that in the non-tumor portion. These findings suggest that the induction of pADPRP may play an important role in the hepatocarcinogenesis induced by peroxisome proliferators.

Heinze R. **A biotest for hepatotoxins using primary rat hepatocytes.** *Phycologia* 1996;35(6 Suppl):89-93.

BIOSIS COPYRIGHT: BIOL ABS. Freshly prepared rat hepatocytes proved highly suitable for screening of cyanobacterial hepatotoxins (microcystins). In particular, short-term exposure to pure microcystins caused severe damage to hepatocytes. The most toxic microcystin variant was microcystin-

LR. In contrast, various cell lines were insensitive to pure microcystin. Algal blooms from a variety of freshwater lakes and rivers were studied and the results, using hepatocyte testing, suggest that two types of toxic cyanobacteria blooms occur. One group exhibited high toxicity to hepatocytes and less or no cytotoxicity to cell lines, and these effects are likely due to microcystins. Another group was toxic to hepatocytes and permanent cells after a longer exposure period, which suggests the presence of other harmful substances.

Oulmi Y, Braunbeck T. **Toxicity of 4-chloroaniline in early life-stages of zebrafish (*Brachydanio rerio*): I. cytopathology of liver and kidney after microinjection.** Arch Environ Contam Toxicol 1996 Mar;30(3):390-402.

In addition to survival and hatching parameters, cytological alterations in liver and kidney of 4- and 6-d old zebrafish larvae (*Brachydanio rerio*) following single microinjection of fertilized eggs at the germ-ring stage with 5, 12.5, and 25 ng 4-chloroaniline/egg were investigated by means of electron microscopy. Whereas survival remained unaffected, microinjection with 4-chloroaniline disturbed hatching of larvae. Hatching was delayed by microinjection of 12.5 ng 4-chloroaniline/egg and above when compared to controls. Cytological investigations revealed ultrastructural changes in both liver and kidney in a dose- and time-dependent fashion. In the liver, major cytopathological changes included fenestration, fragmentation, and vesiculation of the rough endoplasmic reticulum, proliferation of atypical mitochondria, and atypical lysosomes. Furthermore, myelin whorls, lipid inclusions, and cholesterol crystals were increased, whereas glycogen stores were reduced. Renal tubular cells displayed altered brush borders, proliferation of nucleoli, atypical mitochondria, fenestrated, fragmented, and vesiculated RER cisternae, as well as giant lysosomes. Most of these effects indicate cellular dysfunction (e.g., disturbance of lipid metabolism in the liver), whereas others illustrate general cellular stress-responses to chemical aggression. Comparisons of results with those of previous studies based on conventional fish exposure prove the suitability and sensitivity of microinjection bioassays with zebrafish eggs as an alternative to conventional early life-stage tests.

IMMUNOTOXICITY

Arimura M, Yokozeki H, Katayama I, Nakamura T, Masuda M, Nishioka K. **Experimental study for the development of an in vitro test for contact allergens. 2. Comparison of the in vitro sensitization test with the guinea pig maximization test for contact allergens.** Int Arch Allergy Immunol 1998;115 (3):228-34.

We have previously reported an in vitro hapten-specific sensitization method using Pam-212 cells (in vitro sensitization test) to identify the potential effectiveness of contact allergens. In the present study, we conducted comparison studies of 11 allergens and 2 irritants in order to evaluate the method as an alternative predictive test. The guinea pig maximization test (GPMT) was developed based on the test described by Magnusson and Kligman. Our assay was carried out as follows: we treated Pam-212 cells with 13 test chemical solutions, while T cells and macrophages of BALB/c mice were cultured with hapten-conjugated Pam-212 cells for 5 days. After incubation, 10⁵ T cells were stimulated with mitomycin-C-treated spleen cells conjugated with chemicals. Three days later, the [³H]methyl thymidine incorporation was counted. The results of the GPMT were in agreement with those reported in previous studies except for benzocaine. In our GPMT experiments, benzocaine was negative, but it

had been classified as a moderate sensitizer in previous studies. Our assay detected extreme, strong and moderate sensitizers as previously classified by the GPMT. They could be summarized as follows: three of five chemicals classified as moderate sensitizers, and 100% of strong or extreme sensitizers were detected by both the GPMT and the in vitro sensitization test. No irritants showed a positive reaction in our assay. These results support the view that the sensitivity of our in vitro test may be equivalent to that of the GPMT and may be useful as a rapid and objective allergen screening test.

Dearman RJ, Basketter DA, Kimber I. **Selective induction of type 2 cytokines following topical exposure of mice to platinum salts.** Food Chem Toxicol 1998;36(3):199-207.

Repeated topical exposure of BALB/c strain mice to organic chemical respiratory allergens, such as trimellitic anhydride (TMA), or contact allergens such as 2,4-dinitrochlorobenzene (DNCB), provokes characteristic cytokine secretion profiles consistent with the divergent activation of discrete T cell subpopulations. Under such conditions, lymph node cells (LNC) isolated from animals exposed to TMA elaborated comparatively large amounts of the type 2 cytokines interleukin 10 (IL-10) and mitogen-inducible interleukin 4 (IL-4), but only low levels of the type 1 product interferon gamma (IFN-gamma). In contrast, DNCB-activated LNC displayed the converse (type 1) cytokine secretion profile. We have now examined cytokine production induced by topical application to mice of respiratory sensitizing platinum salts; ammonium tetrachloroplatinate II, ammonium hexachloroplatinate IV and cis-dichlorodiammine platinum II. Metal salts were dissolved in dimethyl sulfoxide (DMSO). Cytokine secretion profiles were compared with those elicited following concurrent exposure to TMA or DNCB or to the vehicle acetone:olive oil (AOO) alone. All three platinum salts and TMA stimulated vigorous IL-4 and IL-10 production compared with DNCB-activated LNC; vehicle-stimulated LNC failed to elaborate detectable levels of either cytokine. However, DNCB and the DMSO vehicle provoked substantial IFN-gamma expression, whereas exposure to AOO vehicle resulted in a considerably weaker IFN-gamma response. Levels of this cytokine induced by treatment with respiratory allergens were, in the majority of cases, substantially lower than those observed with the relevant vehicle. Indeed, an inverse dose-response relationship for IFN-gamma expression was exhibited by all three platinum salts, suggestive of the elaboration by platinum salt activated LNC of an inhibitory factor or factors for IFN-gamma. These data suggest that it may be possible to identify those metal salts with respiratory sensitizing potential as a function of induced type 2 cytokine secretion patterns.

Graham C, Rosenkranz HS, Karol MH. **Structure-activity model of chemicals that cause human respiratory sensitization.** Regul Toxicol Pharmacol 1997;26(3):296-306.

We report a structure-activity model of chemicals with the potential to cause respiratory allergy developed through the CASE/MultiCASE systems. Chemicals documented to elicit a decrease in FEV1 of $>$ or $=$ 20% within 24 h of inhalation provocation challenge were used to form a learning set. Additional requirements for inclusion in the learning set were that chemicals had at least two contiguous nonhydrogen atoms and were nonmetallic. Forty chemicals met these criteria. The model identified several structural alerts including the isocyanate functionality ($N = C = O$), primary and secondary amines, substituted aromatic moieties, and distance descriptors. An external data-withholding exercise used to validate the model yielded a sensitivity of 0.95 and a specificity of 0.95. This model is applicable to initial prediction of the sensitizing ability of untested chemicals and may provide mechanistic insight into the process(es) of respiratory sensitization.

Henry JA. **Composition and toxicity of petroleum products and their additives.** Hum Exp Toxicol 1998;17(2):111-23.

BIOSIS COPYRIGHT: BIOL ABS. 1. All petroleum based products are highly complex chemical mixtures. Although almost exclusively composed of hydrocarbons, the composition varies with the crude oil source. 2. Their toxicity for man is generally low but there are exceptions. Although irritancy and sensitization to specific ingredients may be demonstrated in animals, animal experiments are not a reliable indicator of sensitization potential in man. 3. Both product complexity and commercial considerations can make acceptable and meaningful compositional disclosures difficult. A nomenclature system exists which solves these problems. 4. Frame formulations would have some value to poisons centres dealing with petroleum product enquiries. 5. As legislation for the European Union is developed, the balance must be reached between disclosure of the (often confidential) precise chemical composition of products and a practical and useful composition for the guidance of users and medical personnel. This is a key issue with some petroleum products, mainly due to the additives used in them. 6. For several reasons, such as climatic conditions or logistics of supply, the various components, including additives, used in a branded product may vary because the final product composition is determined not by chemistry but by performance in service. 7. Lubricants may contain between 10 and 20% of additives; fuels contain additives only at parts per million levels. However, for both fuels and lubricants, toxicity from additives is rarely a matter of concern.

Raszyk J, Toman M, Gajduskova V, Nezveda K, Ulrich R, Jarosova A, Docekalova H, Salava J, Palac J. **Effects of environmental pollutants on the porcine and bovine immune systems.** Vet Med (Praha) 1997;42(11):313-7.

Immunological examinations were done in 30 fattening pigs from three swine farms (D., M., T.) and 20 dairy cows from one cattle farm (N.) in the district of Hodonin in 1994. At the same time, samples of stable dust deposits collected in the four farms were analysed for the contents of mercury, cadmium, lead, gamma-HCH (lindane), and total DDT and polychlorinated biphenyls (PCB). Decreased blood level of lysozyme (0.92 mg/l) was found in the swine herd D. Ten percent of the pigs in the herd were affected with purulent skin lesions. The concentrations of the pollutants under study in the dust deposits collected in the herd D. were low. An increased concentration of PCB (0.263 mg/kg) in stable dust but no adverse effects on the immune system were found in the swine herd M. Eosinophilia (6.2%), increased extensity of *Ascaris suum* infection (25%) and a high content of lead in stable dust (11.2 mg/kg) were typical of the swine herd T. A high content of lindane (0.139 mg/kg) and an extremely high content of lead (214 mg/kg), which can participate in the immunosuppression of lymphocytes, were found in the cattle herd N. The activity of lymphocytes was depressed in 35% of the cows and the mean value was at the lower limit of the physiological range. Hazardous pollutants can affect the immune system of farm animals and their penetration into the stable environment should be prevented.

Sawada H, Nishii K, Suzuki T, Hasegawa K, Hata T, Nagatsu I, Kreitman RJ, Pastan I, Nagatsu T, Kobayashi K. **Autonomic neuropathy in transgenic mice caused by immunotoxin targeting of the peripheral nervous system.** J Neurosci Res 1998;51(2):162-73.

Autonomic neuropathy in several neurodegenerative disorders results from disturbance in physiological functions of different cell types in the central and peripheral nervous systems. For a clearer

understanding of the etiology and pathogenesis of the autonomic disorders it is necessary to create animal models in which degeneration of the causative neuronal types can be induced. Immunotoxin-mediated cell targeting (IMCT) is a novel transgenic mouse technology for eliminating selective cell types with the cytotoxic activity of a recombinant immunotoxin anti-Tac(Fv)-PE40. In this study we conditionally disrupted peripheral catecholaminergic cells with IMCT to generate a mouse model developing autonomic failure based on primary defects of the sympathetic nervous system. Transgenic mice expressing human interleukin-2 receptor alpha subunit under the control of the dopamine beta-hydroxylase gene promoter were intravenously treated with a proper dose of anti-Tac(Fv)-PE40. The immunotoxin induced a selective loss of the target cells in peripheral tissues of the transgenic mice and an impairment of catecholamine metabolism in the tissues. Targeting of the peripheral catecholaminergic cells resulted in severe and progressive phenotypic abnormalities mainly characterized by cardiac dysfunction, hypoactivity, and hypothermia, which explain development of autonomic neuropathy. Our IMCT strategy is useful for elucidating the involvement of different neuronal types and their interactions in the development and symptom of autonomic disorders.

Vallera DA, Panoskaltsis-Mortari A, Blazar BR. **Renal dysfunction accounts for the dose limiting toxicity of DT390anti-CD3sFv, a potential new recombinant anti-GVHD immunotoxin.** *Protein Eng* 1997;10(9):1071-6.

The toxicity of a highly selective, recombinant fusion immunotoxin, DT390anti-CD3sFv, was examined in mice. The protein was expressed from a hybrid gene in which the single chain Fv of the anti-murine CD3 epsilon antibody cDNA was spliced to truncated diphtheria toxin cDNA. DT390anti-CD3sFv, previously shown to have significant anti-GVHD effects when administered to mice given transplants of allogeneic MHC-disparate donor T cells (Vallera et al., *Blood* 88, 2342-2353, 1996), preferentially localized to kidney and had profound renal toxicity as assessed by histology and serum levels of blood urea nitrogen (BUN), and creatinine. Kidney effects were more severe than liver effects at the maximum tolerated dose (MTD) of 10 microg/day BID given over a six day interval. Toxic injury was attributed in part to the toxin moiety since DT390 administered alone was more toxic than equivalent doses of DT390 given in the context of DT390anti-CD3sFv fusion protein. The presence of anti-CD3sFv ligand reduced toxicity since DT390anti-CD3sFv was twice as toxic to severe combined immunodeficiency disease (scid) mice which do not have CD3epsilon expressing T cells as compared to their normal counterparts. Together, these findings further our understanding of the toxicities limiting the in vivo administration of DT fusion immunotoxins in mice and provide a foundation for future genetic modifications which should be directed at reducing these effects.

Zemanek MG, Pollard SJ, Kenefick SL, Hrudey SE. **Toxicity and mutagenicity of component classes of oils isolated from soils at petroleum- and creosote-contaminated sites.** *J Air Waste Manag Assoc* 1997;47(12):1250-8.

Microtox and Ames bioassays were employed to assess acute toxicity and mutagenicity of water soluble components of class-fractionated oils extracted from one creosote- and four petroleum-contaminated soils. Microtox results revealed that potential acute toxicity resides mainly in the polar class fractions at three sites and indicated potential synergistic and antagonistic effects between compounds in the total extracts at two sites. Ames Salmonella/microsome testing indicated that the polyaromatic fractions at two sites exhibit weak mutagenicity with enzymatic activation, while the polar fractions at two sites are

weakly mutagenic without enzyme activation. Further chemical characterization of the polar and polyaromatic fractions is required to fully assess the potential of health and ecological risks at the creosote-and petroleum-contaminated sites exhibiting these toxic responses.

NEUROTOXICITY

Boyes WK, Dourson ML, Patterson J, Tilson HA, Sette WF, Macphail RC, Li AA, O'Donoghue JL. **EPA's neurotoxicity risk assessment guidelines.** *Fundam Appl Toxicol* 1997;40(2):175-84.

The proposed Neurotoxicity Risk Assessment Guidelines (U.S. EPA, 1995c Fed. Reg. 60(192), 52032-52056) of the U.S. Environmental Protection Agency (EPA) were the subject of a workshop at the 1997 Meeting of the Society of Toxicology. The workshop considered the role of guidelines in the risk assessment process, the primary features, scientific basis, and implications of the guidelines for EPA program offices, as well as for industrial neurotoxicologists from the perspectives of both pesticides and toxic substances regulation. The U.S. National Academy of Sciences (NAS, 1983, Risk Assessment in the Federal Government: Managing the Process) established a framework for distinguishing risk management from risk assessment, the latter being the result of integrating hazard identification, hazard characterization, and exposure assessment data. The guidelines are intended to establish operating principles that will be used when examining data in a risk assessment context. The proposed neurotoxicity risk assessment guidelines provide a conceptual framework for deciding whether or not a chemically induced effect can be considered to be evidence of neurotoxicity. Topics in the proposed guidelines include structural and functional effects, dose-response and -duration considerations, and relationships between effects. Among the issues that must be considered are the multiplicity of chemical effects, the levels of biological organization in the nervous system, and the tests, measurements, and protocols used. Judgment of the adversity of an effect depends heavily on the amount and types of data available. The attribution of a chemically induced effect to an action on the nervous system depends on several factors such as the quality of the study, the nature of the outcome, dose-response and time-response relationships, and the possible involvement of nonneural factors. The guidelines will also serve as a reference for those conducting neurotoxicity testing, as well as establish a consistent approach to neurotoxicity risk assessment by regulators. Extending this approach through international harmonization would be advantageous to the development of products for a worldwide market. Thus, both risk assessors and regulated industries have a large stake in the guidelines to provide a framework that will lead to accurate risk assessment decisions.

Chang JY, Phelan KD, Chavis JA. **Neurotoxicity of 25-OH-cholesterol on sympathetic neurons.** *Brain Res Bull* 1998;45(6):615-22.

Cultured rat sympathetic neurons derived from postnatal rat superior cervical ganglia (SCG) were used to compare the neurotoxicity of several cholesterol oxides. The cholesterol oxides tested included: 7-beta-OH-, 7-keto-, 19-OH-, 22(R)-OH-, 22(S)-OH-, and 25-OH-cholesterol. These agents caused an acute as well as a delayed toxicity in sympathetic neurons with 25-OH-cholesterol appearing to be the most toxic. A time-dependent experiment indicated that 25-OH-cholesterol at 4 microg/ml (10 microM) was able to kill 50% of the cells in 36 h. Morphological studies indicate that most of the cells do not exhibit a structural change similar to that observed in neuronal programmed cell death. Whole-cell patch clamp recording of untreated controls and 25-OH-cholesterol (2 microg/ml)-treated cells indicated that

this toxicity was not accompanied by significant changes in voltage-dependent calcium channel activity. A number of pharmacological agents including ethylene glycolbis (beta-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA), cycloheximide, KCl, vitamin E, and methyl-beta-cyclodextrin were able to prevent the 25-OH-cholesterol-induced cell death to various degrees. These results suggest that, in addition to causing pathological changes in cells directly involved in atherosclerosis, cholesterol oxides may induce neurotoxicity in sympathetic neurons.

Chen Q, Olney JW, Lukasiewicz PD, Almlı T, Romano C. **Ca²⁺-independent excitotoxic neurodegeneration in isolated retina, an intact neural net: a role for Cl⁻ and inhibitory transmitters.** *Mol Pharmacol* 1998;53(3):564-72.

Rapidly triggered excitotoxic cell death is widely thought to be due to excessive influx of extracellular Ca²⁺, primarily through the N-methyl-D-aspartate subtype of glutamate receptor. By devising conditions that permit the maintenance of isolated retina in the absence of Ca²⁺, it has become technically feasible to test the dependence of excitotoxic neurodegeneration in this intact neural system on extracellular Ca²⁺. Using biochemical, Ca²⁺ imaging, and electrophysiological techniques, we found that (1) rapidly triggered excitotoxic cell death in this system occurs independently of both extracellular Ca²⁺ and increases in intracellular Ca²⁺; (2) this cell death is highly dependent on extracellular Cl⁻; and (3) lethal Cl⁻ entry occurs by multiple paths, but a significant fraction occurs through pathologically activated gamma-aminobutyric acid and glycine receptors. These results emphasize the importance of Ca²⁺-independent mechanisms and the role that local transmitter circuitry plays in excitotoxic cell death.

Cometto-Muniz JE, Cain WS, Abraham MH. **Nasal pungency and odor of homologous aldehydes and carboxylic acids.** *Exp Brain Res* 1998;118(2):180-8.

Airborne substances can stimulate both the olfactory and the trigeminal nerve in the nose, giving rise to odor and pungent (irritant) sensations, respectively. Nose, eye, and throat irritation constitute common adverse effects in indoor environments. We measured odor and nasal pungency thresholds for homologous aliphatic aldehydes (butanal through octanal) and carboxylic acids (formic, acetic, butanoic, hexanoic, and octanoic). Nasal pungency was measured in subjects lacking olfaction (i.e., anosmics) to avoid odor biases. Similar to other homologous series, odor and pungency thresholds declined (i.e., sensory potency increased) with increasing carbon chain length. A previously derived quantitative structure-activity relationship (QSAR) based on solvation energies predicted all nasal pungency thresholds, except for acetic acid, implying that a key step in the mechanism for threshold pungency involves transfer of the inhaled substance from the vapor phase to the receptive biological phase. In contrast, acetic acid - with a pungency threshold lower than predicted - is likely to produce threshold pungency through direct chemical reaction with the mucosa. Both in the series studied here and in those studied previously, we reach a member at longer chain-lengths beyond which pungency fades. The evidence suggests a biological cut-off, presumably based upon molecular size, across the various series.

De Girolamo LA, Hargreaves AJ, Billett EE. **Axon outgrowth and neurofilament protein expression in mouse neuroblastoma cells exposed to the neurotoxin MPTP.** *Biochem Soc Trans* 1997;25(4):575.

Frey K, Kilbourn M, Robinson T. **Reduced striatal vesicular monoamine transporters after neurotoxic, but not after behaviorally-sensitizing doses of methamphetamine.** *Eur J Pharmacol*

1997;334(2-3):273-9.

Prior studies indicate long-term reductions of striatal dopaminergic markers after sustained, high dose methamphetamine exposures in vivo, suggesting a neurotoxic effect. We have reported lack of regulation of vesicular monoamine transporter type-2 expression, as opposed to other markers of striatal dopaminergic terminals, under conditions that alter dopaminergic transmission without synaptic terminal losses. In the present study, we evaluated the vesicular monoamine transporter and the neuronal membrane dopamine transporter in rat striata after in vivo exposure to neurotoxic or to intermittent, low dose (behaviorally-sensitizing, non-neurotoxic) methamphetamine administrations. Vesicular monoamine transporter binding was measured by autoradiography of (+)-[3H]dihydrotetrabenazine, the active isomer of (+/-)-[3H]dihydrotetrabenazine. (+)-Dihydrotetrabenazine bound to a homogeneous population of striatal sites in controls with a K_d of 1.5 nM and a B_{max} of 3.8 fmol/microg protein. Neurotoxic methamphetamine treatment reduced both striatal vesicular monoamine transporter (-26%) and dopamine transporter (-39%) bindings. There were no changes after the non-neurotoxic treatment regimen. The vesicular monoamine transporter may thus be a valuable marker in the further clinical study of psychostimulant drug neurotoxicity.

Hand CM, Vender JR, Black P. **Chemotherapy in experimental brain tumor, part 1: in vitro colorimetric MTT assay.** J Neurooncol 1998;36(1):1-6.

This study concerns the use of the MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide] colorimetric assay to evaluate the chemosensitivity of the C6 rat glioma cell line to a panel of twelve chemotherapeutic agents. In a previous study of in vitro chemosensitivity of human glioma cell lines, the present authors found a range of sensitivities of the respective cell lines to a panel of chemotherapeutic agents [1]. We then devised an experimental strategy to begin an in vivo evaluation of the correlation between in vitro chemosensitivity and clinical response in an in vivo animal model, such as the model employing the C6 rat glioma cell line. As a step towards utilizing the C6 rat glioma in vivo model, we carried out the present study (Part 1) to determine the correspondence between chemosensitivity to human glioma cell lines and the rat C6 glioma cell line. If a correspondence were to be found, this would enable experimental use of the C6 tumor model for in vivo testing of chemotherapeutic agents. As reported in this paper (Part 1), a correspondence was found, suggesting that the C6 rat glioma represents a suitable model of human glioma for chemotherapeutic studies. This finding served as a basis for proceeding with an in vivo study of chemotherapeutic efficacy which is the subject of a companion report [2].

Harry GJ, Billingsley M, Bruinink A, Campbell IL, Classen W, Dorman DC, Galli C, Ray D, Smith RA, Tilson HA. **In vitro techniques for the assessment of neurotoxicity.** Environ Health Perspect 1998;106 (Suppl 1):131-58.

Risk assessment is a process often divided into the following steps: a) hazard identification, b) dose-response assessment, c) exposure assessment, and d) risk characterization. Regulatory toxicity studies usually are aimed at providing data for the first two steps. Human case reports, environmental research, and in vitro studies may also be used to identify or to further characterize a toxic hazard. In this report the strengths and limitations of in vitro techniques are discussed in light of their usefulness to identify neurotoxic hazards, as well as for the subsequent dose-response assessment. Because of the complexity of the nervous system, multiple functions of individual cells, and our limited knowledge of biochemical

processes involved in neurotoxicity, it is not known how well any in vitro system would recapitulate the in vivo system. Thus, it would be difficult to design an in vitro test battery to replace in vivo test systems. In vitro systems are well suited to the study of biological processes in a more isolated context and have been most successfully used to elucidate mechanisms of toxicity, identify target cells of neurotoxicity, and delineate the development and intricate cellular changes induced by neurotoxicants. Both biochemical and morphological end points can be used, but many of the end points used can be altered by pharmacological actions as well as toxicity. Therefore, for many of these end points it is difficult or impossible to set a criterion that allows one to differentiate between a pharmacological and a neurotoxic effect. For the process of risk assessment such a discrimination is central. Therefore, end points used to determine potential neurotoxicity of a compound have to be carefully selected and evaluated with respect to their potential to discriminate between an adverse neurotoxic effect and a pharmacologic effect. It is obvious that for in vitro neurotoxicity studies the primary end points that can be used are those affected through specific mechanisms of neurotoxicity. For example, in vitro systems may be useful for certain structurally defined compounds and mechanisms of toxicity, such as organophosphorus compounds and delayed neuropathy, for which target cells and the biochemical processes involved in the neurotoxicity are well known. For other compounds and the different types of neurotoxicity, a mechanism of toxicity needs to be identified first. Once identified, by either in vivo or in vitro methods, a system can be developed to detect and to evaluate predictive ability for the type of in vivo neurotoxicity produced. Therefore, in vitro tests have their greatest potential in providing information on basic mechanistic processes in order to refine specific experimental questions to be addressed in the whole animal.

Kim CS, Binienda Z, Sandberg JA. **Construction of a physiologically based pharmacokinetic model for 2,4-dichlorophenoxyacetic acid dosimetry in the developing rabbit brain.** *Toxicol Appl Pharmacol* 1996 Feb;136(2):250-9.

A physiologically based pharmacokinetic (PBPK) model that describes the kinetics of organic anions by using 2,4-dichlorophenoxyacetic (2,4-D) as a representative compound was constructed for the developing rabbit brain at near-term pregnancy (Gestation Day 30). The model consisted of brain, body, and venous and arterial compartments for the mother which were linked to the fetus by a placenta. Maternal brain compartments in the model were brain plasma, cerebrospinal fluid (CSF), and brain tissue including hypothalamus, caudate nucleus, hippocampus, forebrain, brainstem, and cerebellum. The fetus consisted of brain, body, amniotic fluid, and venous and arterial compartments. The maternal body had both a central and a deep compartment; the fetal body had only one compartment. Maternal blood flow to the fetus was modeled as blood flowing to the placenta, where it was equilibrated before it reached the fetus. The brain uptake was membrane-limited by the blood-brain barrier, with saturable clearance from the CSF into the venous blood by the choroid plexus in both fetus and mother. The model was used to compare concentrations of 2,4-D in maternal and fetal brain, maternal and fetal plasma, and amniotic fluid over time with experimental data from pregnant rabbits given 2,4-D intravenously (1, 10, or 40 mg/kg). The model adequately simulated the 2-hr time course of 2,4-D concentrations in both mother and fetus. With continued development, this generic PBPK model should be a useful tool for evaluating the safety of organic acid neurotoxicants in the developing brain.

Konno K, Miwa A, Takayama H, Hisada M, Itagaki Y, Naoki H, Yasuhara T, Kawai N. **alpha-**

Pompilidotoxin (alpha-PMTX), a novel neurotoxin from the venom of a solitary wasp, facilitates transmission in the crustacean neuromuscular synapse. *Neurosci Lett* 1997;238(3):99-102.

BIOSIS COPYRIGHT: BIOL ABS. A new neurotoxin, named alpha-pompilidotoxin (alpha-PMTX) has been found in the venom of the solitary wasp *Anoplius safnariensis*. In the neuromuscular synapse of the lobster walking leg preparation, alpha-PMTX (10-100 μ M) caused great enhancement of both the excitatory and inhibitory postsynaptic potentials. Recordings of the excitatory post synaptic currents (EPSCs) at the synaptic sites showed that alpha-PMTX reversibly and dose-dependently potentiates EPSCs. alpha-PMTX may act primarily on the presynaptic membrane but the mode of action of the toxin is clearly different from other known facilitatory neurotoxins, such as alpha-latrotoxin, apamin or charybdotoxin. This novel toxin will serve as a useful tool in the research field of neuroscience.

Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, Morgan TE, Rozovsky I, Trommer B, Viola KL, et al. **Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins.** *Proc Natl Acad Sci U S A* 1998;95(11):6448-53.

Abeta1-42 is a self-associating peptide whose neurotoxic derivatives are thought to play a role in Alzheimer's pathogenesis. Neurotoxicity of amyloid beta protein (Abeta) has been attributed to its fibrillar forms, but experiments presented here characterize neurotoxins that assemble when fibril formation is inhibited. These neurotoxins comprise small diffusible Abeta oligomers (referred to as ADDLs, for Abeta-derived diffusible ligands), which were found to kill mature neurons in organotypic central nervous system cultures at nanomolar concentrations. At cell surfaces, ADDLs bound to trypsin-sensitive sites and surface-derived tryptic peptides blocked binding and afforded neuroprotection. Germ-line knockout of Fyn, a protein tyrosine kinase linked to apoptosis and elevated in Alzheimer's disease, also was neuroprotective. Remarkably, neurological dysfunction evoked by ADDLs occurred well in advance of cellular degeneration. Without lag, and despite retention of evoked action potentials, ADDLs inhibited hippocampal long-term potentiation, indicating an immediate impact on signal transduction. We hypothesize that impaired synaptic plasticity and associated memory dysfunction during early stage Alzheimer's disease and severe cellular degeneration and dementia during end stage could be caused by the biphasic impact of Abeta-derived diffusible ligands acting upon particular neural signal transduction pathways.

Leanza G, Stanzani S. **Extensive and permanent motoneuron loss in the rat lumbar spinal cord following neurotoxic lesion at birth: morphological evidence.** *Neurosci Lett* 1998;244(2):89-92.

The efficacy of the neurotoxic lectin volkensin to induce motoneuron loss in the lumbar spinal cord was investigated at different time-points following unilateral injection into the medial gastrocnemius muscle of newborn (postnatal day 1 (PD 1)) animals, using retrograde fluorescent neuron labelling and histochemical procedures to evaluate the extent of the toxin-induced depletion, in comparison with the effects produced by neonatal crushing of the sciatic nerve. The results show that very low doses (2.0 ng) of volkensin intramuscularly can produce extensive (about 90%) and long-lasting (up to at least 8 months post-lesion) motoneuronal loss in the lumbar spinal cord, whose magnitude is higher than that observed following mechanical injury of the developing peripheral nerve (50-60%). Volkensin-induced motoneuronal depletion may therefore represent a useful model for experimental studies aimed at functional cell replacement in the immature spinal cord.

Maruyama W, Takahashi T, Naoi M. **(-)-Deprenyl protects human dopaminergic neuroblastoma SH-SY5Y cells from apoptosis induced by peroxynitrite and nitric oxide.** *J Neurochem* 1998;70(6):2510-5.

In Parkinson's disease the cell death of dopamine neurons has been proposed to be mediated by an apoptotic death process, in which nitric oxide may be involved. This article reports the induction of apoptosis by nitric oxide and peroxynitrite in human dopaminergic neuroblastoma SH-SY5Y cells and the antiapoptotic activity of (-)-deprenyl. After the cells were treated with a nitric oxide donor, NOR-4, or a peroxynitrite donor, SIN-1, DNA damage was quantitatively studied using a single-cell gel electrophoresis (comet) assay. NOR-4 and SIN-1 induced DNA damage dose-dependently. Cycloheximide and alkaline treatment of the cells prevented the DNA damage, indicating that the damage is apoptotic and that it depends on the intracellular signal transduction. Superoxide dismutase and the antioxidants reduced glutathione and alpha-tocopherol protected the cells from the DNA damage. (-)-Deprenyl protected the cells from the DNA damage induced by nitric oxide or peroxynitrite almost completely. The protection by (-)-deprenyl was significant even after it was washed from the cells, indicating that (-)-deprenyl may activate the intracellular system against apoptosis. These results suggest that (-)-deprenyl or related compounds may be neuroprotective to dopamine neurons through its antiapoptotic activity.

Padeh Y. **The predator and the prey: elapid neurotoxins and nicotinic acetylcholine receptors.** *Einstein Q J Biol Med* 1997;14(3):63-72.

BIOSIS COPYRIGHT: BIOL ABS. Elapid neurotoxins are polypeptides, which include many different short and long chain neurotoxins, as well as several cytotoxins. The cytotoxins share certain structural features with the short and long neurotoxins, but function via a different, not fully understood mechanism. All the others have been shown to share certain properties which allow them to compete successfully with acetylcholine (ACh) for the binding sites on the nicotinic acetylcholine receptor (nAChR). Five different properties have been proposed: mimicking the agonist, having a concave secondary structure, being flexible, having an open beta-sheet interface for extended beta-sheet formation, and having either packets or hydrophobic/aromatic residues that can tightly bind aromatic residues of the nAChR. All of these properties probably play a role in the binding of neurotoxins to the nAChR but, based on current evidence, the crucial property is the ability to bind aromatic residues of the nAChR. Although there is disagreement as to the exact location of this region on neurotoxins, two possibilities include a tryptophan-binding pocket on loop III or a highly conserved Trp residue (28 or 29) that resides in the concavity of the neurotoxins. There is significant evidence that points to the residues on the cc subunits of the nAChR that participate in and are critical for the previous binding mechanism, namely, Pro-194, Trp-187, and Tyr-189. This can best be seen in *Nature*, where these residues are highly conserved in susceptible animals, but have been universally substituted by non-hydrophobic/non-aromatic residues in resistant animals. Finally, it has been hypothesized that neurotoxins fail to activate the nAChR (even though they have been demonstrated to bind tightly to the ACh binding site) because they are too large and do not allow the intra- and inter-subunit conformational changes and rotations to take place. Normally when ACh binds to the nAChR receptor, a conformational change in the alpha subunits (which is where the binding sites are located) results in a rotation and tilting of the adjacent subunits. This causes the leucine ring (which has been theorized to be the primary barrier to ion passage through the receptor) comprised of invariant Leu-251 residues on the

M2 helices of each of the subunits to dissociate. In addition, previously hidden hydrophilic residues are introduced to the inner surface of the channel, facilitating the passage of ions.

Prasad KN, La Rosa FG, Prasad JE. **Prostaglandins act as neurotoxin for differentiated neuroblastoma cells in culture and increase levels of ubiquitin and beta-amyloid.** *In Vitro Cell Dev Biol Anim* 1998;34(3):265-74.

Although chronic inflammatory reactions have been proposed to cause neuronal degeneration associated with Alzheimer's disease (AD), the role of prostaglandins (PGs), one of the secretory products of inflammatory reactions, in degeneration of nerve cells has not been studied. Our initial observation that PGE1-induced differentiated neuroblastoma (NB) cells degenerate in vitro more rapidly than those induced by RO20-1724, an inhibitor of cyclic nucleotide phosphodiesterase, has led us to postulate that PGs act as a neurotoxin. This study has further investigated the effects of PGs on differentiated NB cells in culture. Results showed that PGA1 was more effective than PGE1 in causing degeneration of differentiated NB cells as shown by the cytoplasmic vacuolation and fragmentation of soma, nuclei, and neurites. Because increased levels of ubiquitin and beta-amyloid have been implicated in causing neuronal degeneration, we studied the effects of PGs on the levels of these proteins during degeneration of NB cells in vitro by an immunostaining technique, using primary antibodies to ubiquitin and beta-amyloid. Results showed that PGs increased the intracellular levels of ubiquitin and beta-amyloid prior to degeneration, whereas the degenerated NB cells had negligible levels of these proteins. These data suggest that PGs act as external neurotoxic signals which increase levels of ubiquitin and beta-amyloid that represent one of the intracellular signals for initiating degeneration of nerve cells.

Pungercar J, Vucemilo N, Faure G, Bon C, Verheij HM, Gubensek F, Krizaj I. **Ammodytin L, an inactive phospholipase A2 homologue with myotoxicity in mice, binds to the presynaptic acceptor of the beta-neurotoxic ammodytotoxin C in Torpedo: an indication for a phospholipase A2 activity-independent mechanism of action of beta-neurotoxins in fish?** *Biochem Biophys Res Commun* 1998;244(2):514-8. A Ser48 phospholipase A2-homologue, ammodytin L, which is myotoxic in mammals and devoid of any phospholipase A2 activity, completely inhibits the specific binding of the neurotoxic phospholipase A2, ammodytotoxin C, to fish presynaptic membranes from *Torpedo marmorata* electric organ. In cross-linking experiments, 125I-ammodytin L labels the same membrane proteins as 125I-ammodytotoxin C (70, 38.5-57.4 and 19.7 kDa). The formation of these adducts is completely prevented by the presence of ammodytotoxin C but not of a non-toxic phospholipase A2, ammodytin I2. A chimeric phospholipase A2, constructed by associating the N-terminal half of ammodytotoxin to the C-terminal half of ammodytin L, possesses a low, but significant phospholipase A2 activity, however it is not toxic to mice, probably due to abolition of the specific neuronal acceptor binding in mammals. Nevertheless, the chimeric phospholipase A2 is able to interact with the ammodytotoxin acceptor in *Torpedo marmorata* electric organ. The existence of neuronal acceptors for ammodytin L and for the chimeric phospholipase A2 suggests that they may act as neurotoxins in fish. As ammodytin L does not possess any enzymatic activity it, therefore, appears to be an excellent tool to investigate the mechanism of action of beta-neurotoxins independently of their phospholipase A2 activity.

Rogove AD, Tsirka SE. **Neurotoxic responses by microglia elicited by excitotoxic injury in the mouse hippocampus.** *Curr Biol* 1998;8(1):19-25.

BACKGROUND: Injury to the brain induces dramatic local changes in gene expression, cellular morphology and behavior. Activation of microglial cells occurs as an early event after central nervous system (CNS) injury, but it has not been determined whether such activation plays a causal role in neuronal death. We have investigated this question using an excitotoxin-mediated brain injury model system, in conjunction with an endogenous peptide factor (macrophage/microglial inhibiting factor, MIF) that ablates microglial contribution to the cascade. **RESULTS:** Using MIF, we inhibited the microglial activation that normally follows excitotoxic injury. In cell culture studies, we found that such inhibition blocked the rapid release of microglia-derived tissue plasminogen activator (tPA), an extracellular serine protease made by both neurons and microglia, which we had previously identified as mediating a critical step in excitotoxin-induced neuronal death. Finally, infusion of MIF into the mouse brain prior to excitotoxic insult resulted in the protection of neurons from cell death. **CONCLUSIONS:** Our results demonstrate that microglia undertake a neurotoxic role when excitotoxic injury occurs in the CNS. They also suggest that the tPA released from microglia has a critical role in triggering neurodegeneration.

Vorhees CV. **Methods for detecting long-term CNS dysfunction after prenatal exposure to neurotoxins.** Drug Chem Toxicol 1997;20(4):387-99.

BIOSIS COPYRIGHT: BIOL ABS. Current U.S. Environmental Protection Agency regulatory guidelines for developmental neurotoxicity emphasize functional categories such as motor activity, auditory startle, and learning and memory. A single test of some simple form of learning and memory is accepted to meet the latter category. The rationale for this emphasis has been that sensitive and reliable methods for assessing complex learning and memory are either not available or are too burdensome, and that insufficient data exist to endorse one approach over another. There has been little discussion of the fact that learning and memory is not a single identifiable functional category and no single test can assess all types of learning and memory. Three methods for assessing complex learning and memory are presented that assess two different types of learning and memory, are relatively efficient to conduct, and are sensitive to several known neurobehavioral teratogens. The tests are a 9-unit multiple-T swimming maze and the Morris and Barnes mazes. The first of these assesses sequential learning, while the latter two assess spatial learning. A description of each test is provided, along with procedures for their use, and data exemplifying effects obtained using developmental exposure to phenytoin, methamphetamine, and MDMA. It is argued that multiple tests of learning and memory are required to ascertain cognitive deficits; something no single method can accomplish. Methods for acoustic startle are also presented.

Yu B, Shinnick-Gallagher P. **Corticotropin-releasing factor increases dihydropyridine- and neurotoxin-resistant calcium currents in neurons of the central amygdala.** J Pharmacol Exp Ther 1998;284(1):170-9.

Corticotropin-releasing factor (CRF) is an important mediator of stress responses in the brain, and CRF receptors and CRF-containing neurons and terminals are located within the central nucleus of the amygdala (CeA). CeA neurons possess multiple types of Ca⁺⁺ channels, including L, N and Q types and a current resistant to saturating concentrations of dihydropyridine and neurotoxin antagonists. In this study, we used whole-cell patch-clamp techniques to study the effects of CRF on whole-cell Ca⁺⁺ current (ICa) in acutely dissociated CeA neurons and determine components of the current affected. CRF (1-400 nM) increased the peak of the ICa in approximately 50% of the CeA neurons recorded. In

the remaining neurons, CRF had little effect. The CRF-induced increase in the ICa was concentration dependent and the estimated EC50 value was 14.9 nM. CRF (50 nM) increased the peak ICa by 25 +/- 5% (n = 9). CRF produced an increase in both the transient and the steady state current but did not shift the peak of the current-voltage relationship. CRF did not affect the voltage dependence of activation and inactivation, and the CRF effect on ICas was not significantly different when the neuron was held at -80 or -40 mV. The competitive CRF receptor antagonist (alpha-helical CRF9-41, 3 microM) blocked the CRF-induced increase in ICa, suggesting that the effect of CRF is receptor mediated. CRF (50 nM) enhanced the ICa (20 +/- 3%) in the presence of saturating concentrations of the L-type blocker nimodipine and neurotoxin N- and Q-type blockers omega-conotoxin GVIA and omega-conotoxin MVIIC. We conclude that CRF increased, through a receptor mechanism, dihydropyridine- and neurotoxin-resistant current(s) in CeA neurons.

OCULAR TOXICITY

Boatright JH, Borst DE, Peoples JW, Bruno J, Edwards CL, Si JS, Nickerson JM. **A major cis activator of the IRBP gene contains CRX-binding and Ret-1/PCE-I elements.** *Mol Vis* 1997;3:15.

PURPOSE: Interphotoreceptor retinoid binding protein (IRBP) is expressed exclusively and to high levels in photoreceptive cells. This study was an attempt to delineate the minimal regulated control region of the murine IRBP promoter involved in this expression pattern. **METHODS:** Fragments of the mouse IRBP 5' flanking region were tested for promoter activity in transient transfections of embryonic chick retina cells in primary culture. Electrophoretic mobility shift assays were used to identify specific cis-acting DNA elements within these fragments. **RESULTS:** Nested deletion analysis of a 1783 bp fragment of the murine IRBP 5' flanking region shows that high promoter activity is maintained with truncated fragments as short as 70 bp 5' to transcription start, but is lost with truncation to 45 bases. The 1783 bp promoter is active in cultures of retina cells but not brain cells or fibroblasts. The 70 bp fragment is active in retina and brain cells but not fibroblasts. Within retina cell cultures, the 1783 bp fragment is active in photoreceptor-like and amorphous or unidentifiable cells whereas the 70 bp is additionally active in multipolar neuron-like cells. The -70 to -45 interval contains Ret-1/PCE-I (AATTAG in the IRBP gene), a proposed retina-specific consensus sequence cis element, and a same-strand reversed copy of this sequence, GATTAA, the consensus binding element of the photoreceptor-specific trans-acting factor CRX. Mutation of either element suppresses promoter activity. Paralleling promoter tissue-specificity, the -70 to -45 fragment binds a sequence-specific protein complex found in retina and brain extracts but not fibroblasts. Mutation of both or either element inhibits this binding. **CONCLUSIONS:** These data suggest that a trans-acting complex binds a cis-element in the -70 to -45 sequence. This binding fully activates transcription but confers only partial tissue-specificity to IRBP gene expression.

Dickerson JE Jr, Dotzel E, Clark AF. **Steroid-induced cataract: new perspective from in vitro and lens culture studies.** *Exp Eye Res* 1997;65(4):507-16.

The prevailing view regarding the mechanism of steroid cataract formation holds that glucocorticoids are covalently bound to lens proteins resulting in destabilization of the protein structure allowing further modification (i.e. oxidation) leading to cataract. Alternative hypotheses (e.g. that cataracts result from glucocorticoid receptor mediated effects) have been difficult to test since protein binding does in fact

occur for many cataractogenic steroids. A glucocorticoid lacking the typical glucocorticoid hydroxy group at C21 (fluorometholone, FML), other steroids which can bind to proteins but lack glucocorticoid activity, and a glucocorticoid antagonist (RU486) have been utilized to discriminate between these two hypotheses. Purified bovine beta-crystallin incubated with three different 3H-steroids, dexamethasone (Dex), aldosterone or progesterone demonstrated that the C-21 hydroxyl group is not essential for steroid binding. Progesterone (with no C-21 OH) bound to the greatest extent. Pretreatment of the protein with aspirin to acetylate the free protein amino groups blocked this binding, demonstrating the probability of a Schiff base mechanism. Lens culture studies with the same three radiolabeled steroids demonstrated much the same result. Rat lenses cultured for 48 hr-11 days, demonstrated that loss of GSH is an early and significant effect of several glucocorticoids (Dex, prednisolone and FML) but is not seen with other non-glucocorticoid steroids. However, none of the steroids tested consistently produced lenticular opacification (i.e. cataracts) in this in vitro system, nor did they alter rubidium transport. We suggest that a mechanism other than covalent binding of steroids to lens proteins is responsible for glucocorticoid induced cataracts because: (1) non-glucocorticoids were demonstrated to bind lens proteins as well or better than the glucocorticoid Dex and (2) only glucocorticoids, and not other steroids, lowered lens reduced glutathione content which has been demonstrated to be associated with other forms of cataract.

Erdinger L, Kirsch F, Sonntag HG. [**Irritating effects of disinfection by-products in swimming pools**]. Zentralbl Hyg Umweltmed 1998;200(5-6):491-503. (Ger)

BIOSIS COPYRIGHT: BIOL ABS. Compounds which can occur as disinfection by-products (DBPs) in swimming pool water were examined for their mucous membrane irritating potential. Previous studies using the rabbit eye test (Draize test) have shown that the irritating potential of typical concentrations of free and combined chlorine are insufficient to explain the degree of eye irritation that can result from exposure to swimming pool water. Other DBPs which may be responsible for eye irritation include halogenated carboxyl compounds (HCC's) which act as precursors during the formation of chloroform. In this study, a modified HET-CAM Test (Hens Egg Test at the Chorion Allantois Membrane) has been used to investigate the mucous membrane irritating effects of HCCs. Some of the compounds tested were found to have a significantly increased irritating effect when compared to a chlorine/chloramine mixture of the same concentration, several mixtures of HCCs were even more active at lower concentrations than single compounds. However, the irritating effects of individual compounds as well as of mixtures of HCC's were not sufficiently intense to allow the identification of those compounds specifically responsible for the overall observed increase in irritation. HCCs were therefore tested in the presence of aqueous chlorine solution. When combined with aqueous chlorine, a number of compounds exhibited significantly enhanced effects. Our results show that the eye irritating effects of low concentrations of DBPs can be investigated using a modified HET-CAM assay. Moreover, results obtained using this assay suggest that the mucous membrane irritating potential of swimming pool water is a consequence of the effects and synergistic action of a number of DBPs in the presence of chlorine. Further work should be carried out in order to establish an indicator for eye irritating effects of swimming pool water.

Gettings SD, Lordo RA, Feder PI, Hintze KL. **A comparison of low volume, Draize and in vitro eye irritation test data. II. Oil/water emulsions.** Food Chem Toxicol 1998;36(1):47-59.

The second phase in a series of investigations of the relationship between low volume eye test (LVET)

data, Draize eye irritation test data, and comparable data from in vitro eye irritation test protocols is presented. These investigations utilize Draize eye test and in vitro endpoint data generated previously as part of the CTFA Evaluation of Alternatives Program. LVET data were generated de novo using the same 18 representative oil/water based personal-care formulations. In general, these formulations were minimally to mildly irritating; only three were classified as moderate eye irritants. The linear correlation between maximum average score as determined by the Draize test (MAS) and the LVET (LVET-MAS) was 0.85; LVET-MAS values were typically about half the corresponding MAS values. Comparison of in vitro assay performance with that of the LVET was determined by statistical analysis of the relationship between LVET-MAS and each in vitro endpoint. Regression modelling was the primary means of enabling such a comparison, the objective being to predict LVET-MAS for a given test material (and to place upper and lower 95% prediction bounds on the range in which the LVET-MAS is anticipated to fall with high probability) based on observation of an in vitro score for that material. The degree of confidence in prediction is quantified in terms of the relative widths of prediction intervals constructed about the fitted regression curves. Sixteen endpoints were shown to have the greatest agreement with the LVET (all but two were selected for modelling when compared with the Draize procedure). While the lower maximum average scores values (compared with the Draize test) in the LVET led to lower variability in LVET-MAS compared to MAS, the upper and lower bounds on predicted LVET-MAS values conditional on observed in vitro scores were still wide. Because there was overlap in the range of scores determined by the prediction bounds for many formulations, each of the selected endpoints was frequently unable to distinguish between test formulations in terms of statistically different predicted LVET-MAS values. In summary, none of the in vitro endpoints evaluated were able to reliably predict values of LVET-MAS among the set of oil/water emulsions considered here.

Gettings SD, Lordo RA, Feder PI, Hintze KL. **A comparison of low volume, Draize and in vitro eye irritation test data. III. Surfactant-based formulations.** Food Chem Toxicol 1998;36(3):209-31.

The third phase in a series of investigations of the relationship between low volume eye test (LVET) data, Draize eye irritation test data, and comparable data from in vitro assay protocols is presented. These investigations utilize Draize eye test and in vitro endpoint data generated previously as part of the CTFA Evaluation of Alternatives Program. LVET data were generated de novo using the same 25 representative surfactant-based personal-care formulations. In general, these formulations were minimally to moderately irritating. The linear correlation between maximum average score as determined by the Draize test (MAS) and the LVET (LVET-MAS) was 0.87; LVET-MAS values were typically about 30% lower than corresponding MAS values. Comparison of in vitro assay performance with that of the LVET was determined by statistical analysis of the relationship between LVET-MAS and in vitro endpoint. Regression modelling was the primary means of enabling such a comparison, the objective being to predict LVET-MAS for a given test material (and to place upper and lower prediction bounds on the range in which the LVET-MAS is anticipated to fall with high probability) based on observation of an in vitro score for that material. The degree of 95% confidence in prediction is quantified in terms of the relative widths of prediction intervals constructed about the fitted regression curves. Twenty in vitro endpoints were shown to have the greatest agreement with the LVET (these endpoints included those with low discordance rates relative to the Draize test) and were therefore selected for regression modelling. Although prediction interval widths tended to be narrower when predicting LVET-MAS compared with predicting MAS, the confidence with which the selected in vitro

endpoints predicted both LVET-MAS and MAS for surfactant-based formulations was greatest when values were close to the lower or upper limits of the observed irritation range (i.e. 95% prediction interval widths were most narrow in these areas). Overall precision of LVET-MAS prediction for surfactant-based formulations was similar to that previously reported for hydroalcoholic formulations and considerably better than was reported for oil/water emulsions.

Maurer JK, Parker RD, Carr GJ. **Ocular irritation: microscopic changes occurring over time in the rat with surfactants of known irritancy.** *Toxicol Pathol* 1998;26(2):217-25.

The pathology of surfactant-induced ocular irritation, especially in the context of accidental human exposures and animal tests used to assess a surfactant's potential ocular irritation, is not well understood. The purpose of this study was to characterize the microscopic changes in rats at 3 hr and on days 1, 2, 3, 4, 7, 14, and 35 following treatment with anionic, cationic, and nonionic surfactants of differing irritancy. The right eye of each rat was treated by placing 10 microliters of a surfactant directly on the cornea. Untreated left eyes served as the controls. At each time point, eyes and eyelids were macroscopically examined and collected for microscopic examination. Macroscopically, the differing levels of irritation were characterized by differences in incidence and magnitude of scores, reflecting involvement of the cornea, conjunctiva, and iris, as well as by the incidence of neovascularization and time to recovery. Microscopically, differences in the area and depth of injury paralleled the differences seen grossly and the relative irritancy of the various surfactants. All surfactants affected the corneal and conjunctival epithelium. All surfactants, except the slightly irritating anionic surfactant, caused corneal stromal changes, with this involvement being proportional to their overall level of irritation. Corneal endothelial cell effects principally occurred with only the severely irritating cationic surfactant. Over time, responses to surfactants of differing irritancy were qualitatively and quantitatively different, and these differences correlated with the extent of initial injury. Qualitative differences in response included presence of keratocyte regeneration, corneal neovascularization, and conjunctivalization of the corneal epithelium with all of the surfactants except the slight irritant. Quantitative differences in response occurred in the extent of epithelial regeneration, edema, and inflammation for surfactants of slight to severe irritancy, and with neovascularization, keratocyte regeneration, and conjunctivalization for surfactants of mild to severe irritancy. These results suggest that by defining initial area and depth of injury associated with an ocular irritant, it may be possible to predict the subsequent response and final outcome. Such an approach would be applicable to the development of mechanistically based in vitro assays.

Ward RK, Mungall S, Carter J, Clothier RH. **Evaluation of tissue culture insert membrane compatibility in the fluorescein leakage assay.** *Toxicol In Vitro* 1997;11(6):761-8.

BIOSIS COPYRIGHT: BIOL ABS. The Fluorescein Leakage (FL) test, a short-term in vitro assay measuring damage on exposure to eye irritants to a transepithelial barrier permeability system, has been assessed as an alternative to the Draize rabbit eye irritation assay in the EU/Home Office international validation trial. The assay is based on the degree of leakage of sodium fluorescein through a confluent layer of Madin-Darby canine kidney epithelial (MDCK) cells grown on a tissue culture insert following exposure to a potential irritant. Several different types of tissue culture inserts with different membrane growth surfaces, pore sizes and pore densities have been employed in the past. Problems with chemical binding and other incompatibilities with insert membranes can affect the sensitivity of MDCK cells.

Cationic surfactants in particular appear to interfere with the passage of fluorescein, leading to underestimation of damage to MDCK cellular function. A blind comparison between MDCK cells cultured on Anopore and the Millicell-HA inserts using the EU/Home Office set of surfactants indicate that the sensitivity of cells cultured on Anopore inserts appeared to correlate more closely with in vivo data. The ability to microscopically evaluate the choice for the FL assay. status of MDCK cultures through Anopore further recommends this membrane as possibly the better choice for the FL assay.

Zeevalk GD, Nicklas WJ. **Activity at the GABA transporter contributes to acute cellular swelling produced by metabolic impairment in retina.** Vision Res 1997;37(24):3463-70.

The role of the GABA transporter in acute toxicity in chick retina due to metabolic inhibition was investigated by the use of several substrate (nipecotic acid, THPO) and nonsubstrate (SKF 89976A, NO711) GABA transport inhibitors. Metabolic stress-induced acute toxicity in the retina is characterized by swelling of distinct populations of retinal neurons and selective release of GABA into the medium. Inhibitor concentrations were based on that needed to attenuate ¹⁴C-GABA uptake at its approximate KM concentration by > or = 70%. Under basal conditions, substrate, but not nonsubstrate, inhibitors increased extracellular GABA, but did not cause histological swelling per se. Under conditions of glycolytic inhibition, nonsubstrate, but not substrate, inhibitors significantly attenuated acute toxicity. Metabolic stress-induced acute toxicity was not altered by the GABA agonist muscimol, nor did muscimol reverse the protective effects of nonsubstrate transport inhibitors, suggesting that an increase in extracellular GABA during metabolic stress was not a component of the acute phase of toxicity. The results indicate that during metabolic inhibition, activity at the GABA transporter contributes to acute cellular swelling.

Zeevalk GD, Nicklas WJ. **Contribution of glial metabolism to neuronal damage caused by partial inhibition of energy metabolism in retina.** Exp Eye Res 1997;65(3):397-405.

Glial cells are relatively resistant to energy impairment, although little is known of the extent to which glial metabolism is affected during partial energy impairment and how this influences neurons. Fluorocitrate has been shown to be a glial specific metabolic inhibitor. Its selective effect on chick retinal Muller cells was verified by measuring incorporation of radiolabel from ³H-acetate and U-¹⁴C-glucose into glutamate and glutamine following exposure of isolated embryonic day 15-18 chick retina to 20 μm fluorocitrate. Fluorocitrate significantly reduced the incorporation of radiolabel from acetate and glucose into glutamine, with less effect on incorporation of label from acetate into glutamate and no reduction of label from glucose into glutamate. The relative specific activity (RSA; ratio of glutamine to glutamate) increased between embryonic day 15 and 18 consistent with the increase in glutamine synthetase activity that occurs in Muller cells at this time in chick retinal development. As with previous findings, mild energy stress produced by inhibiting glycolysis with the general inhibitor iodoacetate (IOA) for up to 45 min, caused acute neuronal damage that was predominately NMDA receptor mediated and occurred in the absence of a net efflux of excitatory amino acids. Acute NMDA-mediated toxicity in this preparation is characterized by the selective damage to amacrine and ganglion cells and quantitatively, by GABA release into the medium. When IOA was combined with fluorocitrate, acute toxicity was potentiated and temporally accelerated. Acute damage was first noted at 15 min, occurred throughout all retinal layers and was accompanied by an overflow of excitatory amino acids at 30 and 45 min. Blocking NMDA receptors with MK-801 during IOA plus fluorocitrate exposure

attenuated the rise in excitatory amino acids and prevented the swelling in neuronal, but not Muller cells. Following incorporation of radiolabel from acetate and glucose into glutamate and glutamine after different times of exposure to IOA showed that while the effects of incorporation of label from glucose were immediate, glutamine synthesis from acetate was preserved for a longer period of time. These findings suggest that during a partial energy impairment, neuronal metabolism is affected to a greater extent than is glial metabolism. Glial cells may play a protective role in this situation, and can delay the onset of acute neuronal damage. Copyright 1997 Academic Press Limited.

PHARMACOKINETIC AND MECHANISTIC STUDIES

Alvinerie M, Escudero E, Sutra JF, Eeckhoutte C, Galtier P. **The pharmacokinetics of moxidectin after oral and subcutaneous administration to sheep.** *Vet Res* 1998;29(2):113-8.

The pharmacokinetic parameters of moxidectin were determined in ten sheep following a single subcutaneous or oral drench at a dose of 0.2 mg.kg⁻¹. The plasma kinetics were best fitted by a two-compartment model. Moxidectin was detected in the plasma at the first sampling time (1 h) and thereafter for at least 60 d. The AUC were similar after both treatments indicating the same bioavailability for the two routes of administration. The oral route was characterized by a higher C_{max} value (28.07 ng.mL⁻¹ than after subcutaneous injection (8.29 ng.mL⁻¹ and by significantly faster absorption as indicated by T_{max} of 0.22 d and 0.88 d for oral and subcutaneous administrations, respectively. The most striking result of this experiment was the longer mean residence time reported for the subcutaneous route, i.e. 16.80 d as compared to 12.55 d for the oral drench. This differences is in agreement with previous studies demonstrating the longer anthelmintic efficacy of the subcutaneous route in comparison with oral administration.

Ashida H, Matsumura F. **Effect of in vivo administered 2,3,7,8-tetrachlorodibenzo-p-dioxin on DNA-binding activities of nuclear transcription factors in liver of guinea pigs.** *J Biochem Mol Toxicol* 1998;12(4):191-204.

To study the long-term effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the DNA-binding activity of nuclear transcription factors; a single dose of TCDD was injected intraperitoneally to male guinea pigs (1 microgram/kg i.p.). The animals were killed after 1, 2, 10, 20, 28, and 40 days, and DNA-binding activities in liver nuclear fraction were assessed through electrophoretic gel mobility shift assay (EMSA). As expected, the nuclear protein binding to dioxin or xenobiotic response element (DRE or XRE) increased as a result of TCDD's action (1-20 days). In addition, protein binding to 32P-labeled activator protein-1 (AP-1) response element (RE) (1-28 days) and activator protein-2 (AP-2) RE (1-28 days) were all increased by the action of TCDD. On the other hand, TCDD treatment significantly lowered the nuclear protein binding to both specific protein-1 (Sp-1) RE and c-MycRE at all time points (1-40 days). In the case of protein binding to 32P-labeled cAMP response element (CRE), we found two groups of binding bands being affected by TCDD. The intensity of the upper band group decreased, and that of the lower band group increased. As for AP-1 proteins, judging by the results of the Western blotting assay, the level of c-Fos increased while that of c-Jun decreased with TCDD treatment both at day 1 and 28. It is known that the rise in AP-1 and AP-2 activities often results in lowering certain cell differentiation signaling messengers in the nucleus. In agreement with this scenario, binding of C/EBP (CCAAT enhancer binding protein) to its response element site was found to be suppressed for 1

through 28 days. Among hormone receptors, TCDD treatment decreased the binding to retinoic acid RE but increased the binding to thyroid hormone RE.

Bailer AJ, Dankovic DA. **An introduction to the use of physiologically based pharmacokinetic models in risk assessment.** Stat Methods Med Res 1997;6(4):341-58.

Many extrapolation issues surface in quantitative risk assessments. The extrapolation from high-dose animal studies to low-dose human exposures is of particular concern. Physiologically based pharmacokinetic (PBPK) models are often proposed as tools to mitigate the problems of extrapolation. These models provide a representation of the disposition, metabolism, and excretion of xenobiotics that are believed to possess the potential of inducing adverse human health responses. Given a model of xenobiotic disposition that is applicable for multiple species and appropriate for nonlinearity of the xenobiotic biotransformation process, better extrapolation may be possible. Unfortunately, the true structure of these models (e.g. number of compartments, type of metabolism, etc.) is seldom known, and attributes of these models (tissue volumes, partition coefficients, etc.) are often experimentally determined and often only central measures of these quantities are reported. We describe the use of PBPK models in risk assessment, the structural and parameter uncertainty in these models, and provide a simple illustration of how these characteristics can be incorporated in a statistical analysis of PBPK models. Additional complexity in the analysis of variability in the models is also outlined. This discussion is illustrated using data from methylene chloride.

Balsamini C, Bedini A, Diamantini G, Spadoni G, Tontini A, Tarzia G, Di Fabio R, Feriani A, Reggiani A, Tedesco G, et al. **(E)-3-(2-(N-phenylcarbamoyl)vinyl)pyrrole-2-carboxylic acid derivatives. A novel class of glycine site antagonists.** J Med Chem 1998;41(6):808-20.

The synthesis and preliminary biological evaluation of novel (E)-3-(2-(N-phenylcarbamoyl)-vinyl) pyrrole-2-carboxylic acids bearing alkyl, acyl, alkoxy, phenyl, and halo substituents at the 4- and 5-positions of the pyrrole ring are reported. These compounds were studied for their in vitro affinity at the strychnine-insensitive glycine-binding site of the N-methyl-D-aspartate (NMDA) receptor complex. In the [³H]glycine binding assay (E)-4,5-dibromo-3-(2-(N-phenylcarbamoyl)vinyl)pyrrole-2-carboxylic acid 6w (pK_i = 7.95 +/- 0.01) and the 4-bromo-5-methyl 6j (pK_i = 7.24 +/- 0.01) and 4,5-dimethyl 6g (pK_i = 6.70 +/- 0.03) analogues were the most active compounds of the series. Qualitative structure-activity analysis points to a negative correlation between bulk of the C-4 and C-5 substituents and affinity which is enhanced by halo-substituents. QSAR analysis by the Hansch descriptors F, R, pi, and MR, on a subset of compounds with pK_i > or = 4, indicates that electron-withdrawing groups at C-4 and C-5 enhance the affinity. Bulk and lipophilicity are also relevant for the substituents at these positions. 6g was found to be a full antagonist (alpha = 0; enhancement of the [³H]TCP binding). The in vivo potency of 6g, 6j, and 6w was evaluated by the inhibition of NMDA-induced convulsions in mice by both the i.v. and po routes; 6w was the most active compound (ED₅₀ = 3 x 10⁻³ (0.8-10) g/kg, i.v. and 30 x 10⁻³ (4.5-61) g/kg, p.o.). The results of this study indicate that the 3,4-disubstitutedpyrrole-2-carboxylate represents a novel template for the design of new glycine antagonists.

Bartroli J, Turmo E, Alguero M, Boncompte E, Vericat ML, Conte L, Ramis J, Merlos M, Garcia-Rafanell J, Forn J. **New azole antifungals. 3. Synthesis and antifungal activity of 3-substituted-4(3H)-quinazolinones.** J Med Chem 1998;41(11):1869-82.

A series ofazole antifungal agents featuring a quinazolinone nucleus have been subjected to studies of structure-activity relationships. In general, these compounds displayed higher in vitro activities against filamentous fungi and shorter half-lives than the structures described in our preceding paper. The most potent products in vitro carried a halogen (or an isostere) at the 7-position of the quinazolinone ring. Using a murine model of systemic candidosis, oral activity was found to be dependent on hydrophobicity, which, in turn, modulated the compound's half-life. The 7-Cl derivative, (1R,2R)-7-chloro-3-[2-(2, 4-difluorophenyl)-2-hydroxy-1-methyl-3-(1H-1,2, 4-triazol-1-yl)propyl]quinazolin-4(3H)-one (20, UR-9825), was selected for further testing due to its high in vitro activity, low toxicity, good pharmacokinetic profile, and ease of obtention. Compound 20 is the (1R,2R) isomer of four possible stereoisomers. The other three isomers were also prepared and tested. The enantiomer (1S,2S) and the (1R,2S) epimer were inactive, whereas the (1S,2R) epimer retained some activity. In vitro 20 was superior to fluconazole, itraconazole, SCH-42427, and TAK-187 and roughly similar to voriconazole and ER-30346. In vivo, 20 was only moderately active in a mouse model of systemic candidosis when administration was limited to the first day. This was attributed to its short half-life in that species ($t_{1/2} = 1$ h po). Protection levels comparable to or higher than those of fluconazole, however, were observed in systemic candidosis models in rat and rabbit, where the half-life of the compound was found to be 6 and 9 h, respectively. Finally, 20 showed excellent protection levels in an immunocompromised rat model of disseminated aspergillosis. The compound showed low toxicity signs when administered to rats at 250 mg/kg qd or at 100 mg/kg bid during 28 days.

Bazzett T, Geiger A, Coppola B, Albin R. **The neuronal NOS inhibitor L-MIN, but not 7-NINA, reduces neurotoxic effects of chronic intrastratial administration of quinolinic acid.** Brain Res 1997;775(1-2):229-32.

Rat striata were exposed to 15 mM quinolinic acid (QUIN), or QUIN plus the nitric oxide synthase inhibitors S-methyl-L-thiocitrulline dihydrochloride (L-MIN) or 7-nitroindazole monosodium salt (7-NINA) for 21 days. Co-administration of 100 microM or 1 mM L-MIN with QUIN significantly reduced lesion volume compared to QUIN alone. Co-administration of 1 microM or 10 microM L-MIN with QUIN had no significant effect. There was no significant effect of 7-NINA co-administered with QUIN compared to QUIN alone. L-MIN reduction of lesion volume supports the contention that neuronal nitric oxide synthase is a mediator of excitotoxic injury.

Bookout RL Jr, Quinn DW, McDougal JN. **Parallel dermal subcompartments for modeling chemical absorption.** SAR QSAR Environ Res 1997;7(1-4):259-79.

Understanding the absorption of chemicals through the skin is of importance to many fields of study. Biologically-based models can be used to simulate the absorption process and predict the rate of absorption and the amount of the chemical in various parts of the body and skin. When these models consist of physiological and biochemical parameters that can be measured, they can be extremely useful. When a model is appropriately validated, the results can be extrapolated across species to predict the effect of human exposure. In this paper we develop two new physiologically-based pharmacokinetic (PBPK) models which predict the concentration of Dibromomethane in the blood of rats after dermal vapor exposure. These two new models expand a previously developed homogeneous skin model by adding parallel skin subcompartments to represent skin appendages and layered subcompartments to represent the distinct layers of the skin. The predictions of these new models match the experimental

data better than the original homogeneous model, as well as being more physiologically descriptive. Sensitivity analysis showed us which parameters were the most sensitive to change and thus revealing the parameters we should be most concerned with measuring. After being properly validated, these models could be a great improvement over previous models in the ability to extrapolate results for different species, doses, and durations.

Borovansky J, Blasko M, Siracky J, Schothorst AA, Smit NP, Pavel S. **Cytotoxic interactions of Zn²⁺ in vitro: melanoma cells are more susceptible than melanocytes.** *Melanoma Res* 1997;7(6):449-53. Previous studies have shown that sensitivity to high extracellular levels of Zn²⁺ is a general feature of cells in vitro and that a prerequisite of the toxic action of zinc is entry into cells via channels that are shared with iron or calcium. As the biochemical and toxicological behaviour of zinc chelate complexes could be different from that of free Zn²⁺, the effect of chelating agents on zinc transport into human melanoma cell lines was tested. EDTAcal and tetracycline reduced the toxic action of zinc ions in vitro, whereas phenytoin and diethyldithiocarbamate potentiated its effects. D-penicillamine, an effective chelator of zinc in vivo, also exerted a protective action in vitro. Comparison of sensitivity to Zn²⁺ in vitro between human melanoma lines and several lines of pigment cells from skin of various origins demonstrated that melanoma cells are killed by zinc ions at concentrations which are only partially toxic for normal pigment cells. This is consistent with the repeatedly observed high uptake of ⁶⁵Zn by melanoma cells.

Bose RN, Fonkeng BS, Moghaddas S, Stroup D. **Mechanisms of DNA damage by chromium(V) carcinogens.** *Nucleic Acids Res* 1998;26(7):1588-96.

BIOSIS COPYRIGHT: BIOL ABS. Reactions of bis(2-ethyl-2-hydroxy-butanato)oxochromate(V) with pUC19 DNA, single-stranded calf thymus DNA (ss-ctDNA), a synthetic oligonucleotide, 5-GATCTATGGACTTACTTCAAGGCCGGGTAATGCTA-3'(35mer), deoxyguanosine and guanine were carried out in Bis-Tris buffer at pH 7.0. The plasmid DNA was only nicked, whereas the single-stranded DNA suffered extensive damage due to oxidation of the ribose moiety. The primary oxidation product was characterized as 5-methylene-2-furanone. Although all four bases (A, C, G and T) were released during the oxidation process, the concentration of guanine exceeds the other three. Orthophosphate and 3'-phosphates were also detected in this reaction. Likewise, the synthetic oligomer exhibits cleavage at all bases with a higher frequency at G sites. This increased cleavage at G sites was more apparent after treating the primary oxidation products with piperidine, which may indicate base oxidation as well. DNA oxidation is shown to proceed through a Cr(V)-DNA intermediate in which chromium(V) is coordinated through the phosphodiester moiety. Two alternative mechanisms for DNA oxidation by oxochromate(V) are proposed to account for formation of 5-methylene-2-furanone, based on hydrogen abstraction or hydride transfer from-the C1' site of the ribose followed by hydration and two successive beta-eliminations. It appears that phosphate coordination is a prerequisite for DNA oxidation, since no reactions between chromium(V) and deoxyguanosine or guanine were observed. Two other additional pathways, hydrogen abstraction from C4' and guanine base oxidation, are also discussed.

Chow JC, Condorelli G, Smith RJ. **Insulin-like growth factor-I receptor internalization regulates signaling via the Shc/mitogen-activated protein kinase pathway, but not the insulin receptor**

substrate-1 pathway. J Biol Chem 1998;273(8):4672-80.

Insulin-like growth factor-I (IGF-I) receptors activate divergent signaling pathways by phosphorylating multiple cellular proteins, including insulin receptor substrate-1 (IRS-1) and the Shc proteins. Following hormone binding, IGF-I receptors cluster into clathrin-coated pits and are internalized via an endocytotic mechanism. This study investigates the relationship between IGF-I receptor internalization and signaling via IRS-1 and Shc. A mutation in the C terminus of the IGF-I receptor decreased both the rate of receptor internalization and IGF-I-stimulated Shc phosphorylation by more than 50%, but did not affect IRS-1 phosphorylation. Low temperature (15 degrees C) decreased IGF-I receptor internalization and completely inhibited Shc phosphorylation. Although receptor and IRS-1 phosphorylation were decreased in accordance with delayed binding kinetics at 15 degrees C, the ratio of IRS-1 to receptor phosphorylation was increased more than 2-fold. Dansylcadaverine decreased receptor internalization and Shc phosphorylation, but did not change receptor or IRS-1 phosphorylation. Consistent with these findings, dansylcadaverine inhibited IGF-I-stimulated Shc-Grb2 association, mitogen-activated protein kinase phosphorylation, and p90 ribosomal S6 kinase activation, but did not affect the association of phosphatidylinositol 3-kinase with IRS-1 or activation of p70 S6 kinase. These data support the concept that Shc/mitogen-activated protein kinase pathway activation requires IGF-I receptor internalization, whereas the IRS-1 pathway is activated by both cell surface and endosomal receptors.

Clewell HJ 3rd, Andersen ME, Wills RJ, Latriano L. **A physiologically based pharmacokinetic model for retinoic acid and its metabolites.** J Am Acad Dermatol 1997 Mar;36(3 Pt 2):S77-85

BACKGROUND: A physiologically based pharmacokinetic (PBPK) model for all-trans-retinoic acid (tretinoin) was developed to provide a coherent description of tretinoin absorption, distribution metabolism, and excretion across species and routes of administration. **OBJECTIVE:** The goal of developing such a model is to provide a measure of internal dose that would be a biologically relevant surrogate for administered dose in assessing human teratogenic risk from topically applied tretinoin emollient cream. **METHODS:** The developed PBPK model included compartments for plasma, liver, gut, intestinal lumen, fat, skin, richly and slowly perfused tissues, placenta, and embryo. Tretinoin metabolism to 13-cis retinoic acid, oxidation, and glucuronidation were incorporated. Dose surrogates, including the maximum plasma concentration (C_{max}) and area under the concentration-versus-time curve were calculated from the model. **RESULTS:** The ability of the model to predict tretinoin pharmacokinetics and to extrapolate across species and routes of administration was tested and validated. Model-derived estimates of dose surrogates demonstrated that the internal exposure to retinoids after topical treatment with 0.05% tretinoin emollient cream is minimal in comparison to that for teratogenic oral doses. The ratio of areas under the curve for total active retinoids after teratogenic oral doses in monkeys versus therapeutic topical doses in human beings, for example, was greater than 450,000 to 1. **CONCLUSION:** For topical application of tretinoin in human beings, detoxification via the glucuronidation pathway predominates, resulting in a much lower internal exposure to active retinoids than was inferred from total radioactivity data. The model predicts that topical application of tretinoin results in an internal exposure that is four to six orders of magnitude lower than a minimally teratogenic dose.

Conover CD, Linberg R, Gilbert CW, Shum KL, Shorr RG. **Effect of polyethylene glycol conjugated bovine hemoglobin in both top-load and exchange transfusion rat models.** Artif Organs 1997;21

(10):1066-75.

The purpose of this study was to determine the effect of the hemoglobin based oxygen carrier, polyethylene glycol conjugated bovine hemoglobin (PEG-Hb) on the physiology of the rat. This study was divided into the following 3 parts: pharmacokinetics, cardiovascular, and histopathology. Pharmacokinetic studies evaluated the PEG-Hb circulatory life and the resultant effect on urine composition. Telemetric intravascular blood pressure probes monitored the heart rate and mean arterial pressure. Renal arterial blood flow was determined by intraoperative perivascular ultrasound. Tissue histology was evaluated for both time and model dependent responses. The mean circulatory half-life of PEG-Hb was 17.7 ± 0.3 h. Proteinuria and hemoglobinuria were greatly reduced with PEG conjugation. PEG-Hb treated rats produced 8.5 times and 49 times less proteinuria and hemoglobinuria, respectively, than unmodified bovine Hb treated animals. The mean arterial pressure (MAP) in PEG-Hb treated rats was insignificantly different from sham controls undergoing a 30% exchange transfusion while dextran caused an initial reduction and bovine Hb produced a prolonged elevation in the MAP. In these same anesthetized rats, PEG-Hb slightly decreased the heart rate while dextran caused an increase and bovine Hb had no effect. In addition, PEG-Hb was able to maintain the renal arterial blood flow while both Ringer's lactate and bovine Hb caused a reduction in the blood flow. Finally, PEG-Hb treated rats showed a dose and time dependent formation of vacuoles within the renal proximal convoluted tubules and splenic macrophages in both top-load and exchange transfusion models, but no other morphological changes. In conclusion, PEG-Hb had a relatively long vascular persistence that did not cause any significant alterations in the urinalysis, cardiovascular function, or tissue histopathology in the rat.

Den Hollander JG, Mouton JW, Verbrugh HA. **Use of pharmacodynamic parameters to predict efficacy of combination therapy by using fractional inhibitory concentration kinetics.** *Antimicrob Agents Chemother* 1998;42(4):744-8.

Combination therapy with antimicrobial agents can be used against bacteria that have reduced susceptibilities to single agents. We studied various tobramycin and ceftazidime dosing regimens against four resistant *Pseudomonas aeruginosa* strains in an in vitro pharmacokinetic model to determine the usability of combination therapy for the treatment of infections due to resistant bacterial strains. For the selection of an optimal dosing regimen it is necessary to determine which pharmacodynamic parameter best predicts efficacy during combination therapy and to find a simple method for susceptibility testing. An easy-to-use, previously described E-test method was evaluated as a test for susceptibility to combination therapy. That test resulted in a MICcombi, which is the MIC of, for example, tobramycin in the presence of ceftazidime. By dividing the tobramycin and ceftazidime concentration by the MICcombi at each time point during the dosing interval, fractional inhibitory concentration (FIC) curves were constructed, and from these curves new pharmacodynamic parameters for combination therapy were calculated (i.e., AUCcombi, Cmax-combi, T>MIC-combi, and T>FIC_i, where AUCcombi, Cmax-combi, T>MIC-combi, and T>FIC_i are the area under the FICcombi curve, the peak concentration of FICcombi, the time that the concentration of the combination is above the MICcombi, and the time above the FIC index, respectively). By stepwise multilinear regression analysis, the pharmacodynamic parameter T>FIC_i proved to be the best predictor of therapeutic efficacy during combination therapy with tobramycin and ceftazidime ($R^2 = 0.6821$; $P < 0.01$). We conclude that for combination therapy with tobramycin and ceftazidime the T>FIC_i is the parameter best predictive of efficacy and that the E-test for susceptibility testing of combination therapy gives promising results. These new

pharmacodynamic parameters for combination therapy promise to provide better insight into the rationale behind combination therapy.

Devito MJ, Diliberto JJ, Ross DG, Menache MG, Birnbaum LS. **Dose-response relationships for polyhalogenated dioxins and dibenzofurans following subchronic treatment in mice. I. CYP1A1 and CYP1A2 enzyme activity in liver, lung, and skin.** Toxicol Appl Pharmacol 1997;147(2):267-80. The dose-response relationships for induction of liver, lung, and skin ethoxyresorufin-O-deethylase (EROD) activity and liver acetanilide-4-hydroxylase (ACOH) activity following subchronic exposure to either 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 1,2,3,7,8-pentachlorodibenzo-p-dioxin, 2,3,7,8-tetrabromodibenzo-p-dioxin, 2,3,7,8-tetrachlorodibenzofuran (TCDF), 1,2,3,7,8-pentachlorodibenzofuran (1-PeCDF), 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF), or octachlorodibenzofuran (OCDF) were determined in female B6C3F1 mice in order to estimate the relative enzyme inducing potency of these chemicals in three different tissues. The relative potencies were calculated based on tissue concentrations as well as administered dose. A dose-dependent induction of EROD activity in liver, lung, and skin and of ACOH activity in liver was found for all seven chemicals. When based on administered dose, the relative potencies for specific congeners did not vary substantially among tissues. The relative potencies for TCDF and 1-PeCDF, congeners which have much shorter half-lives than TCDD, increased for all enzymes when estimated from tissue concentrations. The relative potency of OCDF, which is poorly absorbed, was greater when estimated from tissue concentrations than when estimated from administered dose. 4-PeCDF is highly sequestered in hepatic tissue and when the relative potency was estimated based on tissue concentration, its potency for skin enzyme induction increased. These data indicate that the relative potency of these chemicals is influenced not only by the relative binding affinity to the Ah receptor, but also by differences in pharmacokinetic properties of these chemicals. In addition, it may be useful to derive two sets of toxic equivalency factor values, one used for estimating intake equivalents and the other for estimating tissue equivalents.

Fu LW, Pan QC, Lin GY. [**Compared study of Fura-2/AM assay and MTT assay for screening multidrug resistant modulators**]. Yao Hsueh Hsueh Pao 1997 Jun;32:401-5. (Chi)

IPA COPYRIGHT: ASHP To explore the advantages and disadvantages in screening results of reversing multi-drug resistance by modulators between the Fura-2/AM assay and the MTT assay, 25 compounds having active structures were studied for multidrug resistance reversal activity with both methods. Results suggested that the Fura-2/AM assay may replace the MTT assay in screening on a large scale.

Gebel T. **Arsenic and antimony: comparative approach on mechanistic toxicology.** Chem Biol Interact 1997;107(3):131-44.

A chemico-toxicological similarity between arsenic and antimony exists and their toxicology is often seen. Indeed, both elements possess several common properties, e.g. they are clastogenic but not mutagenic in the trivalent state and they have a carcinogenic potential: trivalent arsenicals are known to be human carcinogens and antimony(III) oxide (by inhalation) has been shown to cause lung cancer in female rats. For years, arsenic has been known to be environmentally toxic. Elevated human exposure to this element, mostly caused by the intake of contaminated tap water, is associated with increased incidences of cancer at various sites. It is still not clear how arsenic compounds exert their genotoxic

effect. It may be connected with an inhibition of DNA repair or the induction of oxidative stress. Little work has been done on the toxicology of antimony as it is less widely present in the environment. There is evidence that in mammals antimony, unlike arsenic, is not detoxified via methylation but it still remains unclear what mechanism is responsible for antimony's genotoxicity. In general, there is little information known about this element to accurately determine its impact on human health. Thus, the aim of this paper is to review current knowledge for future risk assessment and further scientific work.

Hu LA, King SC. **Functional sensitivity of polar surfaces on transmembrane helix 8 and cytoplasmic loop 8-9 of the Escherichia coli GABA (4-aminobutyrate) transporter encoded by gabP: mutagenic analysis of a consensus amphipathic region found in transporters from bacteria to mammals.** *Biochem J* 1998;330(Pt 2):771-6.

The gab permease (GabP) catalyses transport of GABA (4-aminobutyrate) into *Escherichia coli*. Although GabP can recognize and transport many GABA analogues that exhibit activity at GABAergic synapses in the nervous system, the protein domains responsible for these transport and ligand recognition properties have not been studied. Here we report that an amphipathic domain extending through putative transmembrane helix 8 and into the adjoining cytoplasmic region (loop 8-9) contains a critical 20 residue zone within which mutagenesis of polar amino acids has a deleterious effect on [3H] GABA transport activity. This functionally important amphipathic domain is found to be highly conserved in the many APC family transporters that are homologous to GabP. And even though members of the GAT family of GABA transporters from the animal nervous system are not homologous to GabP, an analogous amphipathic structure is found in their loop 8-9 region. These results and observations suggest: (1) that the consensus amphipathic region (CAR) in the putative helix 8 and loop 8-9 region of GabP has functional significance, and (2) that nature has repeatedly used this CAR in transporters from bacteria to mammals.

Hubatsch I, Ridderstrom M, Mannervik B. **Human glutathione transferase A4-4: an alpha class enzyme with high catalytic efficiency in the conjugation of 4-hydroxynonanal and other genotoxic products of lipid peroxidation.** *Biochem J* 1998;330(Pt 1):175-9.

A sequence encoding a novel glutathione transferase, GST A4-4, has been identified in a human fetal brain cDNA library. The protein has been produced in *Escherichia coli* after optimization of the codon usage for high-level heterologous expression. The dimeric protein has a subunit molecular mass of 25704 Da based on the deduced amino acid composition. Human GST A4-4 is a member of the Alpha class but shows only 53% amino acid sequence identity with the major liver enzyme GST A1-1. High catalytic efficiency with 4-hydroxyalkenals and other cytotoxic and mutagenic products of radical reactions and lipid peroxidation is a significant feature of GST A4-4. The k_{cat}/K_m values for 4-hydroxynonanal and 4-hydroxydecenal are $> 3 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$, several orders of magnitude higher than the values for conventional GST substrates. 4-Hydroxynonanal and other reactive electrophiles produced by oxidative metabolism have been linked to aging, atherosclerosis, cataract formation, Parkinson's disease and Alzheimer's disease, as well as other degenerative human conditions, suggesting that human GST A4-4 fulfills an important protective role and that variations in its expression may have significant pathophysiological consequences.

Jan ST, Devanesan PD, Stack DE, Ramanathan R, Byun J, Gross ML, Rogan EG, Cavalieri EL.

Metabolic activation and formation of DNA adducts of hexestrol, a synthetic nonsteroidal carcinogenic estrogen. Chem Res Toxicol 1998;11(5):412-9.

Hexestrol (HES), a synthetic nonsteroidal estrogen, is carcinogenic in Syrian golden hamsters. The major metabolite of HES is its catechol, 3'-OH-HES, which can be metabolically converted to the electrophilic catechol quinone, HES-3',4'-Q, by peroxidases and cytochrome P450. Standard adducts were synthesized by reacting HES-3',4'-Q with dG and dA to produce the adducts 3'-OH-HES-6'(alpha, beta)-N7Gua and HES-3',4'-Q-6'-N6dA, respectively. When HES-3',4'-Q was reacted with calf thymus DNA, 3'-OH-HES-6'(alpha,beta)-N7Gua was identified by HPLC and tandem mass spectrometry as the depurinating adduct, with minor amounts of stable adducts. 3'-OH-HES was bound to DNA after activation by horseradish peroxidase, lactoperoxidase, or rat liver microsomes. The depurinating adduct 3'-OH-HES-6'(alpha, beta)-N7Gua was identified in these systems at levels of 65, 41, and 11 micromol/mol of DNA-P, respectively. Unidentified stable adducts were observed in much lower amounts and were quantified by the ³²P-postlabeling method. Similarly to 3'-OH-HES, the catechol metabolites of the natural steroidal estrogens estrone (E1) and estradiol (E2), namely, 2-OHE1, 4-OHE1, 2-OHE2, and 4-OHE2, can be oxidized to their corresponding quinones by peroxidases and cytochrome P450. The quinones of the carcinogenic 4-OHE1 and 4-OHE2 have chemical and biochemical properties similar to those of HES-3',4'-Q. The results suggest that formation of HES-3',4'-Q may be a critical event in tumor initiation by HES and that HES is an excellent model compound to corroborate the hypothesis that estrogen-3,4-quinones are ultimate carcinogenic metabolites of the natural steroidal estrogens E1 and E2.

Jerca L, Filip F, Alexa ID, Jerca O. [GSH and GSH S-transferase involvement in the bioactivation of xenobiotics. Carcinogen conjugation with reactive metabolites]. Rev Med Chir Soc Med Nat Iasi 1995;99(3-4):23-8. (Rum)

Karlhuber GM, Bauer HC, Eckl PM. Cytotoxic and genotoxic effects of 4-hydroxynonenal in cerebral endothelial cells. Mutat Res 1997;381(2):209-16.

Oxygen free radicals are produced in the central nervous system (CNS) as a consequence of normal physiological metabolic reactions of neuronal cells, but there is evidence accumulating that they are also implicated in the processes leading to a number of pathological changes in the brain. A general mechanism whereby oxygen free radicals induce tissue damage is lipid peroxidation (LPO), which generates a large variety of water-soluble carbonyl compounds. Due to their high reactivity, we focused our investigations on 4-hydroxyalkenals, in particular on 4-hydroxynonenal (HNE), the major 4-hydroxyalkenal. Two phenotypes of cerebral endothelial cells (cECs) were treated with various concentrations of 4-hydroxynonenal and the cyto- and genotoxic effects studied. The cytogenetic endpoints determined were chromosomal aberrations and the induction of micronuclei. Three hours of incubation with HNE induced significantly elevated levels of chromosomal aberrations at concentrations $> \text{ or } = 1 \text{ microM}$ and micronuclei at concentrations $> \text{ or } = 10 \text{ microM}$ in both cEC phenotypes, compared to the controls. Cytotoxicity was observed at a concentration of 50 microM HNE and was significantly higher in the elongated and spindle-shaped cEC phenotype (type II) than in the epithelial cEC phenotype (type I). The results indicate that cECs are affected by HNE even at low concentrations with minor differences between the two cEC phenotypes.

Klopman G₀₁ **The MultiCASE program II. Baseline activity identification algorithm (BAIA).** J Chem

Inf Comput Sci 1998;38(1):78-81.

Kostrzewski P. [**Compartment simulation models and physiologic use in pharmaco- and toxicokinetics**]. Med Pr 1998;49(1):83-92. (Pol)

The pharmaco- and toxicokinetic studies describe the process of absorption, distribution, metabolism and elimination of drugs or chemical compounds in animals and humans. In simple compartmental models, the body is divided into basic compartments, central and peripheral. The central compartment is an equilibrium of arterial and venous blood flows, and the peripheral one is connected to the central compartment through a series of flow rate constants that describe the flow of chemicals in both directions. For instance, we can use the PH/EDSIM software for calculating the constants. The flow of the material from one to the other reflected by vectorial connections of two types of kinetics: linear and Michaelis-Menten (nonlinear). At present, the PB-PK models (physiologically-based pharmacokinetic models), which rely on actual physiological (breath rates, blood flow rates and tissue volumes), biochemical and metabolic parameters, tend to be more commonly used. Tissue groups or compartments that are frequently applied in PB-TK model include organs, muscle, fat tissue and the liver. Tissue compartments are connected by arterial and venous blood flows, and each compartment is characterised by a unique set of differential equations. The flow rate constants that describe that flow of materials from and to the compartments, and the rate of change in the amount of chemical in each compartments are directly proportional to the blood flow rate, tissue solubility and organ value.

Lauwers GY, Kandemir O, Kubilis PS, Scott GV. **Cellular kinetics in Barrett's epithelium carcinogenic sequence: roles of apoptosis, bcl-2 protein, and cellular proliferation**. Mod Pathol 1997;10(12):1201-8.

To gain insight into the neoplastic progression of Barrett's epithelium (BE), we assessed the expression of Ki-67 antigen and bcl-2 protein and the occurrence of apoptosis in metaplastic epithelium with and without regenerative atypia (RA), low-grade dysplasia, and high-grade dysplasia (HGD). To refine our understanding of the epithelial kinetics during the carcinogenic sequence, we performed separate analyses of four different mucosal regions, i.e., surface epithelium, upper and lower crypts, and glands. Expansion of the proliferative zone was noted in dysplasia and to a mild degree in epithelium with RA but not in BE. Expression of bcl-2 protein was seen in the proliferative zone in BE and showed a significant increase in RA but was essentially absent in HGD. Numerous apoptotic nuclei were seen in HGD, decreasing along the cellular gradient from gland to surface. We noted a positive correlation between Ki-67 and bcl-2 in the proliferative zone of BE and RA, whereas a negative correlation was present on the surface of RA. Ki-67 was positively correlated with apoptosis in the lower crypts of HGD. bcl-2 expression was negatively correlated with apoptosis in all regions except the proliferative zone of dysplastic areas. Our findings suggest that overexpression of bcl-2 protein is not an important step in the carcinogenesis of BE. We confirm the upward shift of cellular proliferation in dysplastic epithelia. Apoptosis that is increased in dysplasia might play a significant role in carcinogenesis by restraining increased cellular proliferation.

Lipscomb JC, Garrett CM, Snawder JE. **Use of kinetic and mechanistic data in species extrapolation of bioactivation: Cytochrome P-450 dependent trichloroethylene metabolism at occupationally relevant concentrations**. J Occup Health 1998;40(2):110-7.

BIOSIS COPYRIGHT: BIOL ABS. Trichloroethylene (TRI) is an industrial solvent and environmental contaminant; therefore exposure to TRI occurs in diverse human populations. TRI causes hepatocellular carcinoma in B6C3F1 mice, but not rats; this suggests that TRI may be metabolized differently in the two species. We investigated the metabolism of TRI and the effect of TRI on enzymatic activities indicative of specific cytochrome P450 (CYP) forms in hepatic microsomes from mice, rats and humans. Studies in microsomes estimated Michaelis-Menten kinetic parameters by saturation analysis. Km values were 35.4, 55.5 and 24.6 μM and Vmax values were 5,425, 4,826 and 1,440 pmol/min/mg in pooled mouse, rat and human microsomes, respectively. TRI (1,000 ppm) inhibited CYP2E1 dependent activity in all three species and BROD activity in mice and rats; TRI (1,000 ppm) increased CYP1A1/1A2 activity, and had no effect on CYP2A activity. Inhibition studies with mouse hepatic microsomes demonstrated that TRI was a competitive inhibitor of CYP2E1, with Ki of 50 ppm. TRI noncompetitively inhibited CYP2B-dependent activities in the rat and mouse. Preincubation of microsomes with TRI and NADPH decreased the absorbance of CO-bound CYP in all three species, but the dose-dependence was most evident in mouse hepatic microsomes. These results have quantified the interspecies difference in CYP-dependent TRI bioactivation and indicate that under both equivalent and occupationally relevant (hepatic) exposure conditions the human is at less risk of forming toxic CYP-derived TRI metabolites.

Mahmood I. Development of a limited sampling approach in pharmacokinetic studies: experience with the antiepilepsy drug tiagabine. J Clin Pharmacol 1998;38(4):324-30.

A sparse sampling method is proposed to assess pharmacokinetic parameters after a single dose of the antiepilepsy drug tiagabine. Pharmacokinetic parameters obtained from two different pharmacokinetic studies were compared using sparse sampling (7 blood samples) with extensive sampling (15 to 16 blood samples). The results indicated that sparse blood samples taken at appropriate times can be used to estimate pharmacokinetic parameters as accurately as extensive blood samples. In addition, a limited sampling model (LSM) was developed using samples from 10 subjects at two time points (6 and 8 hours). The model was validated in 40 subjects and provided good population mean estimates of area under the concentration-time curve (AUC) and maximum concentration (Cmax). The sparse sampling method described here can be used to assess pharmacokinetic parameters in drug development provided a prior knowledge of the pharmacokinetics of a drug has been obtained from extensive sampling. Further, the LSM described here may be useful in estimating AUC and Cmax of tiagabine using two samples in clinical settings. The LSM approach described here can also be used to estimate AUC and Cmax of a drug in preclinical toxicokinetic studies without detailed pharmacokinetic studies.

McClellan RO. Use of mechanistic data in assessing human risks from exposure to particles.

Environ Health Perspect 1997;105(Suppl 5):1363-72.

BIOSIS COPYRIGHT: BIOL ABS. The ultimate goal of toxicologic investigations of both natural and man-made fibrous and nonfibrous particles is to provide essential input for the assessment of potential human risks from exposure to these materials. The development of risk assessment procedures for airborne particles has evolved over the years. The earliest assessments for naturally occurring materials used direct human observations and incorporated safety factors to arrive at allowable human exposures. More recently, there has been a need to assess the potential risk associated with production and use of certain man-made materials for which human data are not available or are inadequate. For these

materials, it has been necessary to assess human risks using data obtained from studies conducted in laboratory animals and with cells or tissues. During the last several decades, it has been suggested that data on the mechanisms by which particles cause disease could be used to reduce the uncertainty in estimates of human risks of particle exposures. This article provides comments on the use of mechanistic data in the risk assessment process and suggestions for increasing the successful development and use of mechanistic data in risk assessments conducted in the future.

Michalek JE, Tripathi RC, Kulkarni PM, Gupta PL, Selvael K. **Correction for bias introduced by truncation in pharmacokinetics studies of environmental contaminants.** *Environmetrics* 1998;9(2):165-74.

BIOSIS COPYRIGHT: BIOL ABS. Pharmacokinetic studies of biomarkers for environmental contaminants in humans are generally restricted to a few measurements per subject taken after the initial exposure. Subjects are selected for inclusion in the study if their measured body burden is above a threshold determined by the distribution of the biomarker in a control population. Such selection procedures introduce bias in the ordinary weighted least squares estimate of the decay rate λ caused by the truncation. We show that if the data are conditioned to lie above a line with slope $-\lambda$ on the log scale then the weighted least squares estimate of λ is unbiased. We give an iterative estimation algorithm that produces this unbiased estimate with commercially available software for fitting a repeated measures linear model. The estimate and its efficiency are discussed in the context of a pharmacokinetic study of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Unbiasedness and efficiency are demonstrated with a simulation.

Milesion BE, Chambers JE, Chen WL, Dettbarn W, Ehrich M, Eldefrawi AT, Gaylor DW, Hamernik K, Hodgson E, Karczmar AG, et al. **Common mechanism of toxicity: a case study of organophosphorus pesticides.** *Toxicol Sci* 1998;41(1):8-20.

The Food Quality Protection Act of 1996 (FQPA) requires the EPA to consider available information concerning the cumulative effects of such residues and other substances that have a common mechanism of toxicity ... in establishing, modifying, leaving in effect, or revoking a tolerance for a pesticide chemical residue. This directive raises a number of scientific questions to be answered before the FQPA can be implemented. Among these questions is: What constitutes a common mechanism of toxicity? The ILSI Risk Science Institute (RSI) convened a group of experts to examine this and other scientific questions using the organophosphorus (OP) pesticides as the case study. OP pesticides share some characteristics attributed to compounds that act by a common mechanism, but produce a variety of clinical signs of toxicity not identical for all OP pesticides. The Working Group generated a testable hypothesis, anticholinesterase OP pesticides act by a common mechanism of toxicity, and generated alternative hypotheses that, if true, would cause rejection of the initial hypothesis and provide criteria for subgrouping OP compounds. Some of the alternative hypotheses were rejected outright and the rest were not supported by adequate data. The Working Group concluded that OP pesticides act by a common mechanism of toxicity if they inhibit acetylcholinesterase by phosphorylation and elicit any spectrum of cholinergic effects. An approach similar to that developed for OP pesticides could be used to determine if other classes or groups of pesticides that share structural and toxicological characteristics act by a common mechanism of toxicity or by distinct mechanisms.

Mortensen SR, Brimijoin S, Hooper MJ, Padilla S. **Comparison of the in vitro sensitivity of rat acetylcholinesterase to chlorpyrifos-oxon: what do tissue IC50 values represent?** Toxicol Appl Pharmacol 1998;148(1):46-9.

BIOSIS COPYRIGHT: BIOL ABS. The toxicological literature is replete with studies which have attempted to correlate differences in in vivo sensitivity to anticholinesterases with a common in vitro measure: acetylcholinesterase (AChE) IC50 values. Generally, it is assumed that these IC50 values reflect the intrinsic sensitivity of the AChE molecule to the inhibitor. Our goal was to ascertain whether differences in AChE sensitivity to an organophosphate (i.e., IC50 values) are due to varying properties of the enzyme molecule (i.e., present assumption) or to extrinsic factors. Tissue samples were obtained from immature and adult Long-Evans rats. AChE IC50 values were determined by incubating tissue homogenates with chlorpyrifosoxon (active metabolite of chlorpyrifos, a common organophosphate insecticide) for 30 min at 26 C, and then measuring residual AChE activity. The following IC50 values were noted for postnatal day 4 and adult animals, respectively: brain, 10 nm for both ages; liver, 96 and 527 nm; plasma, 18 and 326 nm. Thus, the apparent sensitivity of AChE was prone to vary dramatically with age and tissue type. In contrast, when AChE was isolated from the same tissues by immunoprecipitation, there were no age- or tissue-related differences (IC50y that IC50 values from a crude homogenate do not measure the true sensitivity of AChE to the inhibitor. Presumably, for chlorpyrifos-oxon, at least, the tissue IC50 values depend greatly on a tissue's propensity to sequester or hydrolyze chlorpyrifos-oxon.

Oyaizu T, Shikata N, Senzaki H, Matsuzawa A, Tsubura A. **Studies on the mechanism of dimethylnitrosamine-induced acute liver injury in mice.** Exp Toxicol Pathol 1997;49(5):375-80. Male and female adult C3H- +/+, C3H-gld/gld.lpr/lpr (gld.lpr) and CBA-lprcg/lprcg (lprcg) mice were given a single i.p. dose of 30 mg/kg dimethylnitrosamine (DMN). Liver tissues were collected from mice killed 6, 12, 24 and 36 hrs post treatment, and the progression of the lesions was characterized morphologically and by the TUNEL method. DMN induced centrilobular hepatic injury accompanied with acute hemorrhage, and all mice died 36 to 48 hrs after the dosing. At 12 hrs after DMN administration, centrilobular hepatocytes revealed nuclear chromatin clumping. At 24 hrs, hepatocyte nuclei became fragmented to form apoptotic cells. Ultrastructurally, chromatin was condensed into a compact granular mass or crescent granular cap at the nuclear periphery. At 36 hrs, the number of apoptotic cells increased and they protruded into the sinusoid or were engulfed by the neighboring hepatocytes. A TUNEL-positive signal preceded the morphological changes and a few normal appearing centrilobular hepatocytes were positive 6 hrs post dosing. Endothelial damage was seen immunohistochemically at 24 hrs by disruption of type IV collagen and factor VIII-related antigen, resulting in massive hemorrhage in the centrilobular to mid zone. No inflammatory reactions were observed throughout the degeneration. The findings indicate that a single i.p. administration of DMN induced severe and fatal toxicity in liver tissues in mice which resembled human fulminant hepatitis. However, as gld-lpr and lprcg mice defective in apoptosis through the Fas system also showed similar severe liver damage, the Fas/Fas ligand system is not involved in DMN-induced liver apoptosis. No other organs or tissues were damaged, and the control mouse liver was intact.

Rao HV, Ginsberg GL. **A physiologically-based pharmacokinetic model assessment of methyl t-butyl ether in groundwater for a bathing and showering determination.** Risk Anal 1997;17(5):583-

98.

Methyl t-butyl ether (MTBE) is a gasoline additive that has appeared in private wells as a result of leaking underground storage tanks. Neurological symptoms (headache, dizziness) have been reported from household use of MTBE-affected water, consistent with animal studies showing acute CNS depression from MTBE exposure. The current research evaluates acute CNS effects during bathing/showering by application of physiologically-based pharmacokinetic (PBPK) techniques to compare internal doses in animal toxicity studies to human exposure scenarios. An additional reference point was the delivered dose associated with the acute Minimum Risk Level (MRL) for MTBE established by the Agency for Toxic Substances and Disease Registry. A PBPK model for MTBE and its principal metabolite, t-butyl alcohol (TBA) was developed and validated against published data in rats and humans. PBPK analysis of animal studies showed that acute CNS toxicity after MTBE exposure can be attributed principally to the parent compound since the metabolite (TBA) internal dose was below that needed for CNS effects. The PBPK model was combined with an exposure model for bathing and showering which integrates inhalation and dermal exposures. This modeling indicated that bathing or showering in water containing MTBE at 1 mg/L would produce brain concentrations approximately 1000-fold below the animal effects level and twofold below brain concentrations associated with the acute MRL. These findings indicate that MTBE water concentrations of 1 mg/L or below are unlikely to trigger acute CNS effects during bathing and showering. However, MTBE's strong odor may be a secondary but deciding factor regarding the suitability of such water for domestic uses.

Reife RA. **Assessing pharmacokinetic and pharmacodynamic interactions in clinical trials of antiepileptic drugs.** *Adv Neurol* 1998;76:95-103.

An understanding of the pharmacokinetic and pharmacodynamic properties of a drug is a basic requirement for its clinical use. The investigations of these properties and their timing are fairly clearly defined in the drug development process. Without fundamental knowledge of the pharmacokinetics and pharmacodynamics of a drug, a physician could not use it appropriately, nor would a regulatory agency be likely to approve its use. Information about the interactions of a new antiepileptic drug with other antiepileptic drugs also aids a physician and is required, in varying degrees, by regulatory agencies. The amount of this information that is needed depends, in part, on the class of drug and the population for which the agent is intended. Because antiepileptic drugs are often used as polytherapy and generally are developed first for this use, their interaction potential must be part of the thought process in their development. The correct time to obtain this information, however, is not clearly defined. The analytic methodology to investigate the pharmacokinetic profile of an NCE exists and is fairly sophisticated. This methodology has enabled the development of study designs to investigate pharmacokinetic interactions. Because the plasma concentration of an antiepileptic drug may be increased or decreased as a result of pharmacokinetic interactions with concomitant antiepileptic drugs, it is of great importance to know about the specific interaction potential of an antiepileptic drug early in its development. Recent studies have confirmed the importance of investigating pharmacokinetic interactions in phase I before proceeding into phases II and III. Without this information, study results are often difficult to interpret; with this knowledge, study designs can be modified to minimize the confounding effect. A methodology exists to investigate the pharmacodynamic effects of an antiepileptic drug at receptors in cell cultures and in animal models of seizures; however, no procedure has been established to evaluate the short-term or immediate clinical pharmacodynamic effect of an antiepileptic drug, as has been done for other

classes of drugs and other diseases. The clinical effect that is sought in trials with antiepileptic drugs is a reduction in seizures with little toxicity. The methodology to investigate the effect of seizure reduction over time has been used repeatedly with minor variations in the development of all the new antiepileptic drugs. However, no study has evaluated the effect of pharmacodynamic interactions among antiepileptic drugs on seizure reduction. Some studies have purported to show an interaction effect on adverse events, and assumptions are made about pharmacodynamic interactions. Although the information regarding pharmacodynamic interactions is important and existing trial designs could evaluate this, there has been no perceived need to carry out such trials. This information is less accessible than pharmacokinetic interaction information. Moreover, pharmacodynamic interactions, as opposed to pharmacokinetic interactions, are probably unidirectional and lead only to increased effects. Although it would be preferable to have this knowledge, an antiepileptic drug can be used effectively without it; over time, the information about pharmacodynamics will be inferred. Thus, conducting pharmacokinetic interaction studies with antiepileptic drugs early in their development as part of phase I is essential, whereas obtaining pharmacodynamic interaction information can be deferred.

Sato S, Tomita I. **Response differences among mouse strains in DNA damage and skin carcinogenicity of 7,12-dimethylbenz[a]anthracene are due to inducible aryl hydrocarbon hydroxylase activity.** Biol Pharm Bull 1998;21(1):90-2.

Strain differences of mice in the induction of DNA damage in peripheral blood cells and skin tumors were investigated using 7,12-dimethylbenz[a]anthracene (DMBA). DMBA-induced DNA damage and skin tumorigenesis were evaluated using the single cell gel electrophoresis (SCGE) assay and 2-stage carcinogenicity study, respectively. DNA damaged cells were markedly increased in the aryl hydrocarbon hydroxylase (AHH)-inducible mice, BALB/c and C57BL/6, as compared with the AHH-noninducible mice, DBA/2, in the SCGE assay. The AHH-inducible mice were more sensitive to DMBA than the AHH-noninducible mice in the 2-stage carcinogenicity study. These results strongly suggest that the genetic capacity to metabolize PAH is associated with the mutagenicity and carcinogenicity of DMBA.

Sharma A, Jusko WJ. **Characterization of four basic models of indirect pharmacodynamic responses.** J Pharmacokinet Biopharm 1996 Dec;24:611-35.

IPA COPYRIGHT: ASHP Four basic models of indirect pharmacodynamic responses were characterized in terms of changing dose, I_{max} or S_{max} , and IC_{50} or SC_{50} to examine the effects of these fundamental drug properties on response profiles; standard pharmacokinetic parameters were used for generating plasma concentration, and response time profiles were generated using computer simulations. Comparisons to theoretical expectations were made. In all 4 models, the maximum response (R_{max}) (inhibition or stimulation) and the time of its occurrence (TR_{max}) were dependent on the model, dose, I_{max} or S_{max} , and IC_{50} or SC_{50} values. An increase in dose or a decrease in IC_{50} or SC_{50} by the same factor produced identical and superimposable pharmacodynamic response patterns in each of the models. Some parameters were nearly proportional to log dose, while others were nonlinear. It was concluded that assessment of expected response signature patterns may be helpful in experimental designs and in assigning appropriate models to pharmacodynamic data.

Shum YY, Huang N, Walter G, Black A, Sekerke C, Chang T, Whitfield LR. **Development, validation,**

and interlaboratory comparison of an HMG-CoA reductase inhibition assay for quantitation of atorvastatin in plasma matrices. *Ther Drug Monit* 1998;20(1):41-9.

An HMG-CoA reductase inhibition assay was developed and validated for quantitation of atorvastatin in human, dog, rat, and mouse plasma. Atorvastatin was isolated from plasma by protein precipitation. Rat-liver microsomes were used to provide the reductase enzyme. The method was validated by assaying calibration standards and quality controls in triplicate on each of the 3 days. A customized computer program was used for data calculation. Quantitation of the assay ranged from 0.36 to 16 ng/ml of atorvastatin in different plasma matrices. Assay precision and accuracy, based on the coefficient of variation and percent relative error, respectively, of quality controls were 10.4% to 14.5% and within +/- 6.25% in human; 4.89% to 10.6% (+/- 8.13%) in dog; 2.68% to 8.62% (+/- 5.00%) in rat; and 3.68% to 8.96% (+/- 5.38%) in mouse plasma. The method has been applied to pharmacokinetic studies of atorvastatin in human and toxicokinetic studies in dog, rat, and mouse after atorvastatin administration. Atorvastatin equivalent concentrations in a set of plasma samples from subjects receiving single and multiple doses of atorvastatin were determined by validated HMG-CoA reductase inhibition assays at four different laboratories. Results were compared using linear regression and concordance correlation statistical procedures. Good agreements among these data indicated that results from different laboratories with the same validated method can be used interchangeably.

Simonian AL, Rainina EI, Wild JR. **A new approach for discriminative detection of organophosphate neurotoxins in the presence of other cholinesterase inhibitors.** *Anal Lett* 1997;30(14):2453-68.

BIOSIS COPYRIGHT: BIOL ABS. A radically new approach for the discriminative determination of various neurotoxins has been developed. This novel biosensor combines a highly sensitive acetylcholinesterase (AChE) biosensor with immobilized organophosphate hydrolase (OPH). The value of the new concept was demonstrated by the discrimination between carbamate and organophosphorus pesticides. It was shown that the response of traditional AChE-based biosensor to mixed samples containing paraoxon and carbofuran was not simply additive, and the measured concentrations of these pesticides were very different from their real concentrations. This combined OPH/AChE system was able to improve the accuracy of the AChE-based biosensor and to uniquely distinguish paraoxon in mixed solutions containing carbofuran. The presented approach promises a new perspective for real world analyses and opens a new area of discriminative determination of various species in multicomponent solutions.

Singh SB, Beard WA, Hingerty BE, Wilson SH, Broyde S. **Interactions between DNA polymerase beta and the major covalent adduct of the carcinogen (+)-anti-benzo[a]pyrene diol epoxide with DNA at a primer-template junction.** *Biochemistry* 1998;37(3):878-84.

A molecular dynamics simulation has been carried out with DNA polymerase beta (beta pol) complexed with a DNA primer-template. The templating guanine at the polymerase active site was covalently modified by the carcinogenic metabolite of benzo[a]pyrene, (+)-anti-benzo[a]pyrene diol epoxide, to form the major (+)-trans-anti-benzo[a]pyrene diol epoxide covalent adduct. Thus, the benzo[a]pyrenyl moiety (BP) is situated in the single-stranded template at the junction between double- and single-stranded DNA. The starting structure was based on the X-ray crystal structure of the rat beta pol primer-template and ddCTP complex [Pelletier, H., Sawaya, M. R., Kumar, A., Wilson, S. H., and Kraut, J.

(1994) Science 264, 1891-1903]. During the simulation, the BP and its attached templating guanine rearrange to form a structure in which the BP is closer to parallel with the adjacent base pair. In addition, the templating attached guanine is displaced toward the major groove side and access to its Watson-Crick edge is partly obstructed. This structure is stabilized, in part, by new hydrogen bonds between the BP and beta pol Asn279 and Arg283. These residues are within hydrogen bonding distance to the incoming ddCTP and templating guanine, respectively, in the crystal structure of the beta pol ternary complex. Site-directed mutagenesis has confirmed their role in dNTP binding, discrimination, and catalytic efficiency [Beard, W. A., Osheroff, W. P., Prasad, R., Sawaya, M. R., Jaju, M., Wood, T. G., Kraut, J., Kunkel, T. A., and Wilson, S. H. (1996) J. Biol. Chem. 271, 12141-12144]. The predominant biological effect of the BP is DNA polymerase blockage. Consistent with this biological effect, the computed structure suggests the possibility that the BP's main deleterious impact on DNA synthesis might result at least in part from its specific interactions with key polymerase side chains. Moreover, relatively modest movement of BP and its attached guanine, with some concomitant enzyme motion, is necessary to relieve the obstruction and permit the observed rare incorporation of a dATP opposite the guanine lesion.

Smith WA, Arif JM, Gupta RC. **Effect of cancer chemopreventive agents on microsome-mediated DNA adduction of the breast carcinogen dibenzo[a,l]pyrene.** Mutat Res 1998;412(3):307-14.

Due to the large and expanding number of potential cancer chemopreventive agents, there is an increasing need for short term tests to study the efficacy and mechanisms of these agents. In this study, we have employed a microsome-mediated test system to study the effect of several suspected chemopreventive agents on the DNA adduct formation capacity of the potent mammary carcinogen, dibenzo[a,l]pyrene (DBP). Bioactivation of DBP by Aroclor 1254-induced rat liver microsomes in the presence of calf thymus DNA (300 microg/ml) resulted in the formation of one major and six other prominent DNA adducts (324 adducts/10(7) nucleotides). These adducts were previously determined to be deoxyadenosine (dA) and deoxyanosine (dG)-derivatives of both anti- and syn-DBP-11,12-diol-13,14-epoxides (DBPDE). Intervention with ellagic acid, chlorophyllin, benzyl isocyanate (BIC), oltipraz or genistein (150 microM) strongly diminished DBP-DNA adduction by > or = 75%. Linoleic acid, curcumin and butylated hydroxytoluene (BHT) also significantly inhibited DBP DNA adduction (26-46%) while N-acetylcysteine (NAC) had no effect. Moreover, nonenzymatic studies with anti- and syn-DBPDE isomers revealed that chlorophyllin, ellagic acid, BIC and BHT may be inhibiting DBP-DNA adduction in an enzymatic-independent manner since these agents diminished DBPDE-DNA adduction by 30-75%. Genistein, oltipraz and curcumin did not diminish DBPDE-DNA adduction and therefore most likely require the presence of the microsomal subcellular fraction to inhibit DBP-DNA adduction.

Sturgill MG, Brenner DE, August DA. **Augmentation of hepatic doxorubicin extraction with extracorporeal filtration avoids the dose-dependent, nonlinear increase in AUC observed with systemic administration.** Cancer Chemother Pharmacol 1998;41(3):193-200.

PURPOSE: Regional therapy of primary or metastatic liver cancer with low hepatic extraction ratio drugs such as doxorubicin is constrained by development of systemic toxicity. To examine the effect of augmentation of hepatic drug extraction, a swine model of hepatic artery infusion (HAI) with minimally invasive hepatic venous isolation and hepatic venous drug extraction (HVDE) was developed to study the comparative pharmacokinetic profiles of regional and systemically administered doxorubicin.

METHODS: Doxorubicin 0.5-9 mg/kg was administered to 31 pigs over 90 min either by HAI with simultaneous HVDE or by standard systemic vein infusion. Systemic artery and hepatic vein plasma samples were collected periodically (0 to 240 min) for determination of doxorubicin concentrations by high-performance liquid chromatography. Pharmacokinetic profiles were modeled with PCNONLIN 4.2. **RESULTS:** Concentration-time data were best described in all pigs by a two-compartment open model of elimination. Independent of the route of administration, AUC and C_{max} values increased with dose. Mean systemic AUC and C_{max} values were consistently lower with regional administration, with statistically significant decreases at the 0.5 and 3 mg/kg doses, whereas there was no relationship between hepatic vein parameters and route of administration. There was a linear relationship between mean systemic AUC values and dose in pigs receiving doxorubicin via HAI with HVDE, whereas mean systemic AUC values increased exponentially at doses of 5 mg/kg or above with systemic vein administration. **CONCLUSIONS:** Administration of doxorubicin by HAI with simultaneous HVDE significantly decreases systemic exposure in comparison with standard systemic vein drug infusion, and may protect against nonlinear increases in exposure at higher doses.

Sun H, Ette EI, Ludden TM. **On the recording of sample times and parameter estimation from repeated measures pharmacokinetic data.** J Pharmacokinet Biopharm 1996 Dec;24:637-50.

IPA COPYRIGHT: ASHP A prospective simulation study was carried out to evaluate the effect of error in the recording of sampling times on the accuracy and precision of population parameter estimates from repeated measures pharmacokinetic data; a 2 compartment model with intravenous (IV) bolus inputs was assumed. Random and systematic errors in sampling times ranging from 5-50% using profile randomized design were introduced. Sampling times were simulated in EXCEL while concentration data simulation and analysis were done in NONMEM. The effect of error in sampling times was studied at levels of variability ranging from 15-45% for a drug assumed to be dosed at its elimination half-life. One hundred replicate data sets of 100 subjects each were simulated for each case. Although estimates of clearance (CL) and variability in CL were robust for most sampling time errors, there was an increase in bias and imprecision in parameter estimation as intersubject variability increased. If the interest lies in parameters other than CL, then population study designs should include procedures for minimizing error in sampling time recording relative to dosing history.

Sundberg K, Johansson AS, Stenberg G, Widersten M, Seidel A, Mannervik B, Jernstrom B.

Differences in the catalytic efficiencies of allelic variants of glutathione transferase P1-1 towards carcinogenic diol epoxides of polycyclic aromatic hydrocarbons. Carcinogenesis 1998;19(3):433-6. Previous studies have identified allelic variants of the human glutathione transferase (GST) Pi gene and showed that the two different encoded proteins with isoleucine (GSTP1-1/I-105) or valine (GSTP1-1/V-105) at position 105, respectively, differ significantly in their catalytic activities with model substrates. Moreover, recent epidemiological studies have demonstrated that individuals differing in the expression of these allelic variants also differ in susceptibility to tumour formation in certain organs, including such in which polycyclic aromatic hydrocarbons (PAH) may be etiological factors. In the present study the catalytic efficiencies (k_{cat}/K_m) of these GSTP1-1 variants were determined with a number of stereoisomeric bay-region diol epoxides, known as the ultimate mutagenic and carcinogenic metabolites of PAH, including those from chrysene, benzo[a]pyrene and dibenz[a,h]anthracene. In addition, GSTP1-1 mutants in which amino residue 105 is alanine (GSTP1-1/A-105) or tryptophan (GSTP1-1/W-105)

have been constructed and characterized. GSTP1-1/V-105 was found to be more active than GSTP1-1/I-105 in conjugation reactions with the bulky diol epoxides of PAH, being up to 3-fold as active towards the anti- and syn-diol epoxide enantiomers with R-absolute configuration at the benzylic oxiranyl carbon. Comparing the four enzyme variants, GSTP1-1/A-105 generally demonstrated the highest k_{cat}/K_m value and GSTP1-1/W-105 the lowest with the anti-diol epoxides. A close correlation was observed between the volume occupied by the amino acid residue at position 105 and the value of k_{cat}/K_m . With the syn-diol epoxides, such a correlation was observed with alanine, valine and isoleucine, whereas tryptophan was associated with increased k_{cat}/K_m values. The mutational replacement of isoleucine with alanine or tryptophan at position 105 did not alter the enantio selectivity of the GSTP1-1 variants compared with the naturally occurring allelic variants GSTP1-1/I-105 and GSTP1-1/V-105. Since the amino acid at position 105 forms part of the substrate binding site (H-site) the effect of increasing bulkiness is expected to cause restricted access of the diol epoxide and proper alignment of the two reactants for efficient glutathionylation. In conclusion, the present study indicates that individuals who are homozygous for the allele GSTP1* B (coding for GSTP1-1/V-105) display a higher susceptibility to malignancy because of other factors than a decreased catalytic efficiency of GSTP1-1/V-105 in the detoxication of carcinogenic diol epoxides of benzo[a]pyrene or structurally related PAH.

Witcher JW, Boudinot FD. **Applications and simulations of a discontinuous oral absorption pharmacokinetic model.** Pharm Res 1996 Nov;13:1720-4.

IPA COPYRIGHT: ASHP A discontinuous oral absorption model was used to fit ranitidine and cimetidine serum concentrations following oral and intravenous administration and to illustrate the effect of various model parameters on plasma drug concentration vs time profiles and bioavailability; plasma concentration vs time data for ranitidine and cimetidine were obtained from the literature. Serum concentrations were well described by the model and parameter estimates were in agreement with literature values.

Worakul N, Robinson JR. **Ocular pharmacokinetics/pharmacodynamics.** Eur J Pharm Biopharm 1997;44(1):71-83.

IPA COPYRIGHT: ASHP An overview of various ocular pharmacokinetic/pharmacodynamic models for different model drugs and drug delivery systems is presented, including a schematic diagram of ocular absorption, ocular penetration routes for the administration of topical ophthalmic drugs, a cylindrical model of the vitreous body of rabbits for analyzing the pharmacokinetics of intravitreal drug delivery, and a comparison of pharmacokinetic factors between rabbit and human eye.

Xu R, Mao B, Amin S, Geacintov NE. **Bending and circularization of site-specific and stereoisomeric carcinogen-DNA adducts.** Biochemistry 1998;37(2):769-78.

The potent tumorigen and mutagen (+)-7(R),8(S)-dihydroxy-9(S), 10(R)-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene ((+)-anti-BPDE) is a metabolite of benzo[a]pyrene that binds predominantly to the exocyclic amino group of guanine residues in DNA in vivo and in vitro. While the (-)-7S,8R,9R,10Senantiomer, (-)-anti-BPDE, also reacts with DNA to form similar covalent N2-deoxyguanosyl adducts, this diol epoxide is nontumorigenic and its mutagenic activities are different from those of (+)-anti-BPDE. In this work, T4 ligase-induced cyclization methods have been employed to demonstrate that the (+)-anti-[BP]-N2-dG lesions (G*) cause significantly greater amounts of bending and circularization of the one-base

overhang undecamer duplex 5'-d(CACAT[G*]TACAC).d(TGTACATGTGG) than the stereoisomeric oligonucleotide duplex with G* = (-)-anti-[BP]-N2-dG. In the case of the (+)-anti-BPDE-modified oligonucleotides, the ratio of circular to linear DNA multimers reaches values of 8-9 for circle contour sizes of 99-121 base pairs, while for the (-)-anti-[BP]-N2-dG-modified DNA this ratio reaches a maximum value of only approximately 1 at 154-176 base pairs. Assuming a planar circle DNA model, the inferred bending angles for 90-92% of the observed circular ligation products range from 30 to 51 degrees per (+)-trans-anti-[BP]-N2-dG lesion and from 20 to 40 degrees per (-)-trans-anti-[BP]-N2-dG lesion. In the case of unmodified DNA, the probability of circular product formation is at least 1 order of magnitude less efficient than in the BPDE-modified sequences and about 90% of the circular products exhibit bending angles in the range of 14 -19 degrees . In the most abundant circular products observed experimentally, the bending angles are 40 degrees and 26 +/- 2 degrees per (+)-anti-[BP]- or (-)-anti-[BP]-modified 11-mer; these values correspond to a net contribution of 21-26 degrees and 5-19 degrees , respectively, to the observed overall bending per lesion. The coexistence of circular DNA molecules of different sizes and, therefore, different average bending angles per lesion, suggest that the lesions induce both torsional flexibility and flexible bends, which permit efficient cyclization, especially in the case of (+)-trans-[BP]-N2-dG adducts. The NMR characteristics of (+)-trans-[BP]-N2-dG lesion in the 11-mer duplex 5'-d(CACAT[G*]TACAC).d(GTGTACATGTG) indicate that all base pairs are intact, except at the underlined base pairs. This suggests a distortion in the normal conformation of the duplex on the 5'-side of the modified guanosine residue, which may be due to bending enhanced base pair opening and bending induced by the bulky carcinogen residue. The implications of base sequence-dependent flexibilities and conformational mobilities of anti-[BP]-N2-dG lesions on DNA replication and mutation are discussed.

Zavros Y, Shulkes A. **Cholecystinin (CCK) regulates somatostatin secretion through both the CCK-A and CCK-B/gastrin receptors in sheep.** J Physiol (Lond) 1997;505(Pt 3):811-21.

1. Cholecystinin (CCK) and gastrin both stimulate gastric somatostatin (SOM) secretion in vitro and thus have the potential to modulate their direct effects on the parietal cell. However, the relative potencies and the mechanisms of action of CCK and gastrin on SOM secretion in vivo have not been determined. 2. The objectives of the present study were to compare the in vivo potencies of the sulphated (s) and non-sulphated (ns) forms of gastrin heptadecapeptide (G-17) and CCK octapeptide (CCK-8) on SOM secretion, and to determine the nature of the receptors involved by repeating the studies in the presence of the CCK-A and CCK-B/gastrin receptor antagonists L-364,718 and L-365,260, respectively. All experiments were performed in the chronically cannulated sheep. 3. Dose-response experiments revealed the following potencies for SOM secretion: G-17s = CCK-8s > G-17 ns >> CCK-8ns. However, based on the plasma levels achieved and a higher metabolic clearance rate (MCR) for CCK, CCK-8s was the most potent. 4. Both the CCK-A and CCK-B/gastrin receptor antagonists suppressed CCK-8s-stimulated SOM output. In contrast, G-17s-stimulated SOM output was inhibited by only the CCK-B/gastrin receptor antagonist. 5. Both receptor antagonists increased basal plasma gastrin and CCK levels. 6. The predominant circulating SOM molecular form after both gastrin and CCK stimulation was SOM-14. 7. In conclusion, the sulphated forms of CCK and gastrin are more potent than the non-sulphated forms. Despite sharing a common biologically active carboxy terminus, CCK stimulates SOM secretion by both the CCK-A and CCK-B/gastrin receptors, while gastrin acts via the CCK-B/gastrin receptor alone. These findings explain in part why CCK is a net inhibitor of gastric acid

secretion in vivo.

Terry KK, Elswick BA, Welsch F, Conolly RB. **A physiologically based pharmacokinetic model describing 2-methoxyacetic acid disposition in the pregnant mouse.** *Occupat Hyg* 1996;2:57-65. A physiologically based pharmacokinetic (PBPK) model was developed in pregnant CD-1 mice to describe the disposition of 2-methoxyacetic acid (2-MAA), the primary metabolite and proximate toxicant derived from the ethylene glycol ether, 2-methoxyethanol (2-ME). The objective was to develop a PBPK model that can be applied to much of the organogenesis stage. Data were collected on gestation day (gd) 8, 11, and 13 (plug positive day = gd 0). Pharmacokinetics and tissue partition coefficients for 2-MAA were determined in maternal plasma and conceptus on gd 8, and in plasma, embryo, and extraembryonic/amniotic fluid (EAF) on gd 11 and 13. For simulation of gd 8 data, the conceptus was described as a single compartment and analyzed as a decidual swelling. On gd 11 and 13, the placenta, embryo, and EAF were explicitly described. Several hypotheses were tested for their ability to predict 2-MAA dosimetry and encoded as alternative models having (a) blood flow limited delivery, (b) pH trapping of ionized 2-MAA, (c) active transport, and (d) reversible binding of 2-MAA. Flow-limited descriptions adequately predicted 2-MAA dosimetry on gd 8. The best simulations of data collected on gd 11 and 13 were, however, obtained with an active transport or a binding model. Since the mechanism by which 2-MAA accumulates into the embryo and EAF remains unknown, these mathematical descriptions are empirical. Further development of this PBPK model will facilitate extrapolations between species and may allow more realistic human risk assessments for the developmental toxicity of 2-ME and related compounds.

PULMONARY TOXICITY

Anderson RC, Anderson JH. **Toxic effects of air freshener emissions.** *Arch Environ Health* 1997;52(6):433-41.

To evaluate whether emissions of a commercial air freshener produced acute toxic effects in a mammalian species, the authors allowed male Swiss-Webster mice to breathe the emissions of one commercial-brand solid air freshener for 1 h. Sensory irritation and pulmonary irritation were evaluated with the ASTM-E-981 test. A computerized version of this test measured the duration of the break at the end of inspiration and the duration of the pause at the end of expiration--two parameters subject to alteration via respiratory effects of airborne toxins. Measurements of expiratory flow velocity indicated changes in airflow limitation. The authors then subjected mice to a functional observational battery, the purpose of which was to probe for changes in nervous system function. Emissions of this air freshener at several concentrations (including concentrations to which many individuals are actually exposed) caused increases in sensory and pulmonary irritation, decreases in airflow velocity, and abnormalities of behavior measured by the functional observational battery score. The test atmosphere was subjected to gas chromatography/mass spectroscopy, and the authors noted the presence of chemicals with known irritant and neurotoxic properties. The Material Safety Data Sheet for the air freshener indicated that there was a potential for toxic effects in humans. The air freshener used in the study did not diminish the effect of other pollutants tested in combination. The results demonstrated that the air freshener may have actually exacerbated indoor air pollution via addition of toxic chemicals to the atmosphere.

Kimbell JS, Gross EA, Richardson RB, Conolly RB, Morgan KT. **Correlation of regional formaldehyde flux predictions with the distribution of formaldehyde-induced squamous metaplasia in F344 rat nasal passages.** *Mutat Res* 1997;380(1-2):143-54.

BIOSIS COPYRIGHT: BIOL ABS. Squamous epithelium lines the nasal vestibule of the rat, rhesus monkey, and human. Respiratory, transitional, and olfactory epithelia line most areas posterior to the nasal vestibule. Inhaled formaldehyde gas induces squamous metaplasia posterior to the nasal vestibule and does not induce lesions in the nasal vestibule in rats and rhesus monkeys, indicating that squamous epithelium is resistant to irritant effects of formaldehyde and that squamous metaplasia may be an adaptive response. If squamous metaplasia is determined by formaldehyde dosimetry rather than by tissue-specific factors, squamous epithelium may be protective by absorbing less formaldehyde than other epithelial types. In a previous study, a three-dimensional, anatomically accurate computational fluid dynamics (CFD) model of the anterior F344 rat nasal passages was used to simulate inspiratory airflow and inhaled formaldehyde transport. The present study consisted of two related parts. First, the rat CFD model was used to test the hypothesis that the distribution of formaldehyde-induced squamous metaplasia is related to the location of high-flux regions posterior to squamous epithelium. Regional formaldehyde flux into nonsquamous epithelium predicted by the CFD model correlated with regional incidence of formaldehyde-induced squamous metaplasia on the airway perimeter of one cross-sectional level of the noses of F344 rats exposed to 10 and 15 ppm formaldehyde gas for 6 months. Formaldehyde flux into nonsquamous epithelium was estimated to vary by an order of magnitude depending on the degree of formaldehyde absorption by squamous epithelium. These results indicate that the degree to which squamous epithelium absorbs formaldehyde strongly affects the rate and extent of the progression of squamous metaplasia with continued exposure to formaldehyde. In the second part of this study, the CFD model was used to predict squamous metaplasia progression. Data needs for verification of this model prediction are considered. These results indicate that information on the permeability of squamous epithelium in rats, monkeys, and humans is important for accurate prediction of uptake in regions posterior to the nasal vestibule.

QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIPS

Blaha L, Damborsky J, Nemeč M. **QSAR for acute toxicity of saturated and unsaturated halogenated aliphatic compounds.** *Chemosphere* 1998;36(6):1345-65.

BIOSIS COPYRIGHT: BIOL ABS. Training-set of 19 compounds - 11 haloalkanes and 8 haloalkenes - was selected from a group of 58 halogenated aliphatic hydrocarbons using statistical experimental design. Strictly defined method was used for preparation of water solutions and toxicity measurements of volatile and poorly water-soluble halogenated aliphatic substances. The acute toxicity expressed as the effective concentrations (EC50) was determined for the compounds in the training set using the Microtox test. The quantitative structure-activity relationships (QSAR) models for haloaliphatic compounds were constructed using the Projection of latent structures method. Size of the molecules was the most important parameter for toxicity of saturated haloaliphatic compounds. This characteristic can be related to accumulation of the haloalkanes in biological membranes or binding to biomacromolecules. Toxicity of 2-chlorobutane was significantly higher than expected from its size. This compound, as the only representative of beta-substituted chloroderivatives in the data set, has probably different mode of action than terminally substituted compounds. Three unsaturated compounds cis-1,2-dichloroethylene,

trans-1,4-dichlorobutene, and cis-1,4-dichlorobutene - displayed similar mode of action to that observed for haloalkanes, while another two haloalkenes 3-chloropropylene, and 2,3-dibromopropylene displayed different - reactive - type of toxicity. The steric parameters had to be complemented by four electronic descriptors for explanation of their high toxicity.

Gute BD, Basak SC. **Predicting acute toxicity (LC50) of benzene derivatives using theoretical molecular descriptors: a hierarchical QSAR approach.** SAR QSAR Environ Res 1997;7(1-4):117-31.

Four classes of theoretical structural parameters, viz., topostructural, topochemical, geometrical and quantum chemical descriptors, have been used in the development of quantitative structure-activity relationship (QSAR) models for a set of sixty-nine benzene derivatives. None of the individual classes of parameters was very effective in predicting toxicity. A hierarchical approach was followed in using a combination of the four classes of indices in QSAR model development. The results show that the hierarchical QSAR approach using the algorithmically derived molecular descriptors can estimate the LC50 values of the benzene derivatives reasonably well.

Gutser UT, Friese J, Heubach JF, Matthiesen T, Selve N, Wilffert B, Gleitz J. **Mode of antinociceptive and toxic action of alkaloids of Aconitum spec..** Naunyn Schmiedebergs Arch Pharmacol 1998;357(1):39-48.

Extracts of the plant *Aconitum spec.* are used in traditional Chinese medicine predominantly as anti-inflammatory and analgesic agents, the latter allegedly equally potent as morphine but without any habit-forming potential. As the only pharmacologically active compounds, the C19 diterpenoid alkaloid aconitine, and some of its derivatives, have been proven to be antinociceptive in different analgesic assays, but the mode of action is unknown. To elucidate the mode of action, ten aconitine-like derivatives were investigated with respect to their affinity for voltage-dependent Na⁺ channels, the action on synaptosomal Na⁺ and Ca²⁺ homeostasis and their antinociceptive, arrhythmogenic and acute toxic properties. Since aconitine is known to bind to site II of Na⁺ channels, we determined the affinity of the aconitine-like derivatives in vitro to synaptosomal membranes by the [³H]-batrachotoxinin-binding assay and their properties on intrasynaptosomal concentrations of free Na⁺ and Ca²⁺ ([Na⁺]_i and [Ca²⁺]_i), both the latter determined fluorometrically with SBFI and Fura-2 respectively. Furthermore, the alkaloids' arrhythmogenic potential was investigated in guinea-pig isolated atria and the antinociceptive action on formalin-induced hyperalgesia and the acute toxic action estimated in mice. The results show that the alkaloids could be divided into at least three groups. The first is characterized by a high affinity to the site II of Na⁺ channels (K_i about 1.2 μM), the ability to enhance [Na⁺]_i and [Ca²⁺]_i (EC₅₀ about 3 μM), a strong arrhythmogenic action that starts at about 30 nM, an antinociceptive effect (ED₅₀ about 0.06 mg/kg) and high acute toxicity (LD₅₀ values about 0.15 mg/kg). To this group belong aconitine, 3-acetylaconitine and hypaconitine. The second group, with lappaconitine as the only member, has an affinity to Na⁺ channels an order of magnitude lower (K_i = 11.5 μM), less acute toxicity (LD₅₀ about 5 mg/kg), and a two orders of magnitude lower antinociceptive action (ED₅₀ about 2.8 mg/kg) and lower cardiotoxicity (bradycardia observed at 3 μM). Additionally, lappaconitine suppresses the increase in [Ca²⁺]_i of aconitine-stimulated synaptosomes and increases the excitation threshold of left atria, indicating an inhibition of Na⁺ channels. The other derivatives, i.e. delcorine, desoxydelcorine, karakoline, lappaconidine, lappaconine

and lycoctonine, belong to the third group, which has hardly any effects. They have a low affinity to Na⁺ channels with K_i values in the millimolar range, show no effect on synaptosomal [Na⁺]_i and [Ca²⁺]_i, no arrhythmogenic potential up to 100 μM, no antinociceptive activity and low toxicity with LD₅₀ values greater than 50 mg/kg. For the investigated alkaloids we suggest two different antinociceptive-like modes of action. Aconitine, hypaconitine and 3-acetylaconitine may induce a block of neuronal conduction by a permanent cell depolarisation, whereas lappaconitine might act like local anaesthetics. However, because of the low LD₅₀/ED₃₀ quotients of 2-6, the antinociceptive-like action of the Aconitum alkaloids seems to reflect severe intoxication rather than a specific antinociceptive action. The structure/activity relationship shows that alkaloids that activate or block Na⁺ channels have a benzoyl ester side chain in the C-14 or C-4 positions respectively, whereas the other compounds lack this group.

Hansch C, Zhang L. **Comparative QSAR: radical toxicity and scavenging. Two different sides of the same coin.** SAR QSAR Environ Res 1995;4(2-3):73-82.

From an analysis of the toxicity of phenols to rat embryos and anilines to embryo fibroblast cells a new type of toxicity is postulated for these classes of compounds. Substituents which increase the electron density on the aromatic ring as estimated by sigma⁺ or epsilon HOMO increase potency. It is postulated that it is the radical form of the phenols and the anilines that accounts for their toxicity. The results are compared with QSAR for radical scavengers and oxidoreductases acting on phenols, anilines and carbazoles.

Huang QG, Song WL, Wang LS. **Quantitative relationship between the physicochemical characteristics as well as genotoxicity of organic pollutants and molecular autocorrelation topological descriptors.** Chemosphere 1997;35(12):2849-55.

BIOSIS COPYRIGHT: BIOL ABS. Autocorrelation topological indices were used as molecular descriptors to study the relationship between physicochemical characteristics as well as genotoxicity of organic pollutants and the molecular structures. Significant quantitative structure-activity relationships were established. Introduction Quantitative structure-activity relationship (QSAR) studies are useful in assessing environmental effects of chemicals, and different mathematical models have been proposed in order to evaluate the link between structures and characteristics of organic pollutants. (1) Various molecular topological descriptors have been widely applied in describing molecular structure. Among them, the molecular connectivity indices, proposed by Kier, are mainly used in the environmental field; while, the autocorrelation topological indices, based on the autocorrelation mathematic function, are often used in a pharmacological SAR study, but play few roles in environmental science (2-5). Because molecular autocorrelation topological indices do not require any restrictive hypothesis on the nature of molecules and can be manipulated conveniently, it appears very promising in physicochemical parameters and ecotoxicological QSAR investigations. In the article, the calculation of autocorrelation topological index was revised slightly based on the conventional method, and the prediction ability was enhanced significantly. The relationship was well established between solubilities in water (S_w) as well as Octanol/Water partition coefficients (K_{ow}) of 55 substituted benzenes and autocorrelation topological indexes. In addition, the genotoxicity of 26 hydrocarbons was studied through micronuclei test, with 20 compounds active, and their MM (20) were deduced. A satisfying regression equation was obtained between the MNI and autocorrelation topological index.

King RD, Srinivasan A. **The discovery of indicator variables for QSAR using inductive logic programming.** J Comput Aided Mol Des 1997;11(6):571-80.

A central problem in forming accurate regression equations in QSAR studies is the selection of appropriate descriptors for the compounds under study. We describe a novel procedure for using inductive logic programming (ILP) to discover new indicator variables (attributes) for QSAR problems, and show that these improve the accuracy of the derived regression equations. ILP techniques have previously been shown to work well on drug design problems where there is a large structural component or where clear comprehensible rules are required. However, ILP techniques have had the disadvantage of only being able to make qualitative predictions (e.g. active, inactive) and not to predict real numbers (regression). We unify ILP and linear regression techniques to give a QSAR method that has the strength of ILP at describing steric structure, with the familiarity and power of linear regression. We evaluated the utility of this new QSAR technique by examining the prediction of biological activity with and without the addition of new structural indicator variables formed by ILP. In three out of five datasets examined the addition of ILP variables produced statistically better results ($P < 0.01$) over the original description. The new ILP variables did not increase the overall complexity of the derived QSAR equations and added insight into possible mechanisms of action. We conclude that ILP can aid in the process of drug design.

Martinez A, Ochoa C, Rodriguez J, Rodriguez M, Castro A, Gonzalez M, Martinez MM. **Comparative molecular field analysis (CoMFA) on (6) + (6) fused pyrazines with nematocide properties.** Quant Struct Activity Relat 1997;16(5):372-6.

BIOSIS COPYRIGHT: BIOL ABS. Quantitative structure-activity relationship (QSAR) studies, using the Comparative Molecular Field Analysis (CoMFA), on a series of 6,7-diarylpteridine derivatives showing nematocide properties have been carried out. The CoMFA model generated in the study has been used to estimate the nematocide activity (MIC₅₀) of seven 6,7-diarylpteridines related to those previously studied. The model is highly predictive for all pteridine derivatives in the test set. Moreover, this model also predicts satisfactorily the nematocide activity of other (6) + (6) fused pyrazines (quinoxalines and pyridopyrazines) which were not represented in the training set. The analysis revealed the importance of steric factors (64.8%) and then the electrostatic ones (35.8%). Most pteridines under study were previously synthesized and tested as nematocide agents. Now, the synthesis and biological evaluation of 6,7-di-(2'-thienyl)4(3H)-thioxo-pteridine 36 are reported.

McKinney JD, Waller CL. **Molecular determinants of hormone mimicry: halogenated aromatic hydrocarbon environmental agents.** J Toxicol Environ Health B Crit Rev 1998;1(1):27-58.

The potential of ostensibly structurally diverse environmental chemicals to modulate endocrine processes in biological systems has been recognized. Difficulty in classifying endocrine system modulators by chemical structure may in large part be due to lack of understanding of mechanisms of action. New developments in understanding nuclear receptor mechanisms of hormone action support a more complex mechanism, possibly involving dimerization/aggregation events leading to multimeric receptor complexes in agonist action. Because of the requirement for high structural specificity in agonist action, it is suggested that most environmental chemicals of concern are likely to function as imperfect hormones with partial agonist-antagonist properties, especially at environmentally realistic concentrations. In the absence of having appropriately placed molecular recognition domains to affect

agonist action, partial agonism-antagonism may be associated with favorable low-energy conformational flexibility and complementary receptor protein flexibility. The halogenated aromatic hydrocarbons are of particular concern as hormone mimics since they often have (1) similar molecular recognition factors but in many cases relatively more flexible structures, (2) similar bulk physico-chemical properties controlling uptake and distribution in biological systems, and (3) are relatively more resistant to metabolism and elimination. Some important molecular reactivity properties underlying thyromimetic and estrogenic actions of some of these chemicals are identified and described in terms of structure-activity relationships (SARs). It is proposed that specificity of hormone action in the nucleus could be associated with differential interaction of ligand-bound receptor dimeric forms with other transcription factors specific to the target cell. The small-molecule ligand can be viewed as playing a central, multifunctional role in nuclear receptor action as an organic unmasking and reclustered agent for critical macromolecules. Evidence is discussed in support of a nuclear heterodimerization model for dioxin and related compound action involving a structural transition mechanism. These models with some molecular detail also have utility in understanding the different structural properties of agonists and antagonists. There would appear to be ample opportunities for environmental chemicals to act as antagonists for multiple receptor systems with little more than anchor-ring similarities in structure. The application of three-dimensional quantitative structure-activity (3D QSAR) models incorporating such structural information should be a useful adjunct for identifying endocrine system modulating chemicals. This data has implications for (1) improved drug design, (2) understanding of chemical interaction toxicity, (3) removing undesirable chemicals from our environment, and (4) reducing their chemical release.

Wang G, Bai N. **Structure-activity relationships for rat and mouse DL50 of miscellaneous alcohols.** *Chemosphere* 1998;36(7):1475-83.

BIOSIS COPYRIGHT: BIOL ABS. Structure-activity relationships between acute toxicities of 95 alcohols to rat and mouse (oral LD50) and four special substructure factors, hydroxyl number, carbon atom number were examined by means of expert system method. The results showed that the expert system based QSAR model was excellent for classification for miscellaneous alcohols (only 9 of them were wrong classified). It was also used to predict the toxicity of other 25 alcohols, and the false prediction rate was only 12%.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Abir R, Ornoy A. **Teratogenic IgG from sera of women with spontaneous abortions seem to induce anomalies and yolk sac damage in rat embryos. A possible method to detect abortions of immunologic origin.** *Am J Reprod Immunol* 1996 Feb;35(2):93-101.

PROBLEM: Spontaneous abortions due to immunological rejection of the embryo may be avoided by immunotherapy with paternal allogeneic leukocytes but there is no appropriate method to detect and differentiate this group of aborters from other groups. METHODS: In previous studies we have demonstrated that in about two-thirds of sera from women with spontaneous abortions the IgG antibodies are responsible (alone or in combination with other factors) for the embryotoxic effects of these sera on cultured rat embryos. We presently cultured 10.5-day-old rat embryos on highly teratogenic serum (high risk serum that induced anomalies in more than 50% of the embryos) from

women with spontaneous abortions, where the IgG fraction was exchanged with IgG from control sera and vice-versa. We studied by Transmission Electron Microscopy (TEM) the extent of yolk sac damage in comparison to the rate of embryonic anomalies. **RESULTS:** In cases where IgG antibodies were teratogenic, embryos cultured in control sera with IgG from high risk sera exhibited ultrastructural yolk sac damage as well as embryonic anomalies, and the yolk sacs cultured in high risk sera with control IgG were normal. In cases in which the IgG exchange did not change the rate of anomalies, as IgG was not teratogenic, yolk sacs from embryos cultured in high risk sera remained damaged, while yolk sacs from embryos cultured in control sera after IgG exchange stayed normal. Although no significant difference in total IgG levels was found between the groups, a higher IgG1 level in sera from women with teratogenic IgG was observed in comparison to control women's sera. The obstetrical history of the women with two or more abortions who took part in our study showed that there were more cases of unknown etiology of the abortion in the women from the high risk group. **CONCLUSIONS:** The serum and the IgG fraction from women with habitual abortions can be tested in whole embryo culture to evaluate the embryonic and yolk sac damage. On this basis it may be possible to detect the women in whom the habitual abortions result from immunological rejection.

Amacher DE, Stadler J, Schomaker SJ, Verseil C. **The comparative testing of eight coded chemicals in the rat limb bud micromass and rat embryo culture systems.** *Altern Lab Anim* 1996 Nov-Dec;24(6):945-52.

When cultured at high density, mesenchymal cells from rat limb buds proliferate and differentiate into chondrocytes. Inhibition of this in vitro chondrogenic process has been used for the preliminary evaluation of teratogenic potential. Alternatively, intact post-implantation rat embryos, maintained in short-term culture, provide a system for the in vitro study of abnormal development not limited to the skeletal system. Both systems isolate the test agent from maternal metabolism and pharmacokinetic restraints. In this study, drug-associated selective inhibition of alcian blue uptake by cartilage proteoglycans, in micromass cultures of limb bud cells prepared from 13-day-old rat embryos, was used to assess teratogenic potential in vitro following exposure for 48 hours to eight coded compounds (acetylsalicylic acid, isoniazid, penicillin G, saccharine, vincristine sulphate, 6-aminonicotinamide, retinoic acid, and amaranth). Following drug exposure, cultures were incubated for another 96 hours, and the cells were then fixed and stained with 0.5% alcian blue. Bound dye was then extracted and quantitated. In parallel cultures, cell viability was measured by neutral red uptake, and protein content was assayed by using the bicinchoninic acid method. Except for retinoic acid and vincristine sulphate, the maximum test concentration was 1000 ug/mL. Inhibition of alcian blue uptake (greater than or equal to 50%) was noted at 0.001 ug/mL vincristine sulphate, 0.5 ug/mL retinoic acid and 5 ug/mL 6-aminonicotinamide, demonstrating that strong teratogens inhibit differentiation in micromass cultures at lower concentrations than those which affect limb cell viability. When the same eight compounds were tested in a 24-hour embryo culture model, dysmorphogenesis was evident at 0.005 ug/mL vincristine sulphate, 0.1 ug/mL retinoic acid and 0.3 ug/mL 6-aminonicotinamide. For the other five chemicals, little or no toxicity was noted up to the maximum test concentration in either model. We conclude that the two test systems, both based on the developing rat embryo, are consistent with each other, and that either of them would be useful for the preliminary screening of potential teratogens.

Amini SA, Dunkley PR, Murdoch RN. **Teratogenic effects of ethanol in the Quackenbush special**

mouse. Drug Alcohol Depend 1996 May;41(1):61-9.

The intragastric exposure of QS mice to alcohol both under short-term (6-day period) (3.0 g/kg, but not 1.5 g/kg, body weight/day through gestation day (GD) 7 to GD 12) and long-term (chronic) (15% ethanol in drinking water beginning several weeks before mating and continuing into pregnancy) conditions reduced the weight, size, and protein content of GD 12 embryos, and the weight of GD 18 embryos. The incidence of brachydactyly with delayed ossification was also significantly greater in embryos chronically exposed to alcohol than in controls (45% vs. 6.7%). The short-term and long-term exposure regimens produced incidences of only 1% and 5.8%, respectively, of forelimb ectrodactyly in GD 18 embryos. It was concluded that alcohol exerts embryo growth retarding effects in pregnant QS mice without inducing a high incidence of skeletal defects. Thus, the QS mouse could serve as an excellent model to resolve the mechanisms whereby alcohol induces pre- and post-natal growth restrictions during pregnancy.

Amwayi PJ, Otiang'a-Owiti GE. **Use of biometric embryonic growth parameters as indicator of exposure to a teratogen.** East Afr Med J 1997 Jan;74(1):6-11.

Disturbances in embryonic growth were studied in 233 foetuses harvested on day 14.5 of gestation, after the administration of various doses of 5 Fluoro-2' deoxyuridine (FUdR) to pregnant mice on day 11.0 of gestation. Measurements of crown-rump length (CRL) and mean wet body weights showed a significant retardation of embryonic growth ($p < 0.001$), following doses of 30, 80 and 100 mg FUdR per kg maternal body weight. Compared to the controls, whole FUdR-treated embryos that had been macerated, cleared and double stained with alcian blue 8GX plus alizarin red S for skeletal anlage, showed that ossification had not commenced in the vertebral bones of tail. All bones in the craniofacial region and limbs including the girdles, were smaller, while there were distortions of the long bones. The severity of the changes were dependent on the concentration of FUdR dose administered. Among the live FUdR-treated foetuses harvested, 95% had mesomelic limb defects. The incidence of delay or prevention of palatal processes elevation was 79%, 49%, 21% and 30% respectively for 0 mg (control), 30 mg, 80 mg and 100 mg FUdR doses. The results show that administration of a teratogenic agent (FUdR) causes retardation of growth which correlates with abnormalities of the secondary palate and limbs. It is proposed that the initial screening of potential teratogenic substances in food, such as preservatives or colourings, may be carried out by monitoring changes in secondary palate and limb development, including biometric growth parameters of an animal model.

Anderson D, Dobrzynska MM, Yu TW, Gandini L, Cordelli E, Spano M. **DNA integrity in human sperm.** Teratog Carcinog Mutagen 1997;17(3):97-102.

Since the 1970s there have been conflicting reports of decreasing sperm counts in man and increasing testicular cancer. There is a hypothetical link between apparent adverse trends in several measures of human reproductive health and exposure to endocrine disrupters. Rodent bioassays are not suited for the large-scale screening of such chemicals because of their costs, complexity, and ethical concerns. Various in vitro assays have been used to examine the effects of these chemicals, but none has directly used semen as one of the target tissues in man. The present study has examined in the alkaline Comet assay in human sperm the effect of two estrogens--beta-estradiol and the phytoestrogen daidzein--and 1,2-epoxybutene, a metabolite of 1,3-butadiene, and compared them with the effects of the known reprotoxin, dibromochloropropane, in two fertile and two infertile frozen sperm samples and two fresh

fertile samples. While differences were detected in the frozen fertile and infertile samples with flow cytometry, in the Comet assay both frozen and fresh samples exposed to the chemicals in vitro from fertile and infertile men produced similar altered responses by comparison with untreated samples. The integrity of DNA is necessary not only for the noncancerous state, but also for the accurate transmission of genetic material to the next generation. Thus this assay may be useful for examination of chemicals in fresh and frozen sperm samples.

Arlen RR, Wells PG. **Inhibition of thalidomide teratogenicity by acetylsalicylic acid: evidence for prostaglandin H synthase-catalyzed bioactivation of thalidomide to a teratogenic reactive intermediate.** *J Pharmacol Exp Ther* 1996 Jun;277(3):1649-58.

Thalidomide is a teratogenic sedative-hypnotic drug that is structurally similar to phenytoin, which is thought to be bioactivated by prostaglandin H synthase (PHS) and other peroxidases to a teratogenic reactive intermediate. The relevance of this mechanism to thalidomide teratogenicity was evaluated in pregnant New Zealand White rabbits treated with thalidomide at 11:00 A.M. on gestational days 8 to 11, with day 0 indicating the time when sperm were observed in the vaginal fluid. Thalidomide (7.5 mg/kg i. v.) produced mainly fetal limb anomalies analogous to those observed in humans. Thalidomide (25-200 mg/kg i.p.), produced a dose-related increase in a spectrum of fetal anomalies, and in postpartum lethality, but did not produce a reliable incidence of limb anomalies. In subsequent studies, pregnant does received the irreversible PHS inhibitor acetylsalicylic acid (ASA), 75 mg/kg i.p., or its vehicle, followed 2 hr later by thalidomide, 7.5 mg/kg i.v., or its vehicle. ASA pretreatment was remarkably embryoprotective, resulting in respective 61.2 and 61.4% decreases in thalidomide-initiated fetal limb anomalies ($P = .002$) and postpartum fetal lethality ($P < .02$), and a small but significant reduction in thalidomide-initiated fetal weight loss. ASA alone did not produce significant embryopathy. These results show that ASA can protect the embryo from thalidomide teratogenicity, suggesting that thalidomide may be bioactivated by PHS to a teratogenic reactive intermediate.

Bantle JA, Finch RA, Burton DT, Fort DJ, Dawson DA, Linder G, Rayburn JR, Hull M, Kumsher-King M, Gaudet-Hull AM, et al. **FETAX interlaboratory validation study: phase III--Part 1 testing.** *J Appl Toxicol* 1996 Nov-Dec;16(6):517-28.

The Frog Embryo Teratogenesis Assay-Xenopus (FETAX) is a 96-h whole embryo developmental toxicity screening assay that can be used in ecotoxicology and in detecting mammalian developmental toxicants when an in vitro metabolic activation system is employed. A standardized American Society for Testing and Materials (ASTM) guide for the conduct of FETAX has been published, along with a companion atlas that aids in embryo staging and identifying malformations. As part of the ASTM process, a three-phase interlaboratory validation study was undertaken to evaluate the repeatability and reliability of FETAX. Seven different participants collaborated in the study. In Phase I, FETAX proved to be more repeatable and reliable than many bioassays. However, some excessive variation was observed in a few laboratories. An initial lack of assay experience by some technicians caused variation. Phase II showed far less intra- and interlaboratory variability than Phase I. Non-teratogens showed the most consistent results, while more variability was observed for the two teratogens tested. Interlaboratory coefficient of variation values for all endpoints ranged from 7.3 to 54.7. Phase III--Part 1, using coded samples and test concentration ranges selected by each laboratory, showed results similar to Phase I. Analysis of the causes of variation suggested that some technicians judged some embryos to

be malformed while others consistently judged similar embryos as normal. Concentration ranges tested by some of the laboratories varied greatly and a new protocol for selecting concentrations for initial testing was written to reduce variation from this source. Testing to date suggests that FETAX is as repeatable and reliable as other standard bioassays.

Berezin V, Kawa A, Bojic U, Foley A, Nau H, Regan C, Edvardsen K, Bock E. **Teratogenic potency of valproate analogues evaluated by quantitative estimation of cellular morphology in vitro.** *Toxicol In Vitro* 1996 Oct;10(5):585-94.

To develop a simple prescreening system for teratogenicity testing, a novel in vitro assay was established using computer assisted microscopy allowing automatic delineation of contours of stained cells and thereby quantitative determination of cellular morphology. The effects of valproic acid (VPA) and analogues with high as well as low teratogenic activities--(as previously determined in vivo)--were used as probes for study of the discrimination power of the in vitro model. VPA, a teratogenic analogue (+/-)-4-en-VPA, and a non-teratogenic analogue (E)-2-en-VPA, as well as the purified (S)- and (R)-enantiomers of 4-yn-VPA (teratogenic and non-teratogenic, respectively), were tested for their effects on cellular morphology of cloned mouse fibroblastoid L-cell lines, neuroblastoma N2a cells, and rat glioma BT4Cn cells, and were found to induce varying increases in cellular area. Furthermore, it was demonstrated that under the chosen conditions the increase in area correlated statistically significantly with the teratogenic potency of the employed compounds. Setting the cellular area of mouse L-cells to 100% under control conditions, the most pronounced effect was observed for (S)-4-yn-VPA (211%, P less than 0.001) followed by VPA (186%, P less than 0.001), 4-en-VPA (169%, P less than 0.001) and non-teratogenic 2-en-VPA (137%, P less than 0.005) and (R)-4-yn-VPA (105%). This effect was independent of the choice of substrata, since it was observed on L-cells grown on plastic, fibronectin, laminin and Matrigel. However, when VPA-treated cells were exposed to an arginyl-glycyl-aspartate (RGD)-containing peptide to test whether VPA treatment was able to modulate RGD-dependent integrin interactions with components of the extracellular matrix, hardly any effect could be observed, whereas control cells readily detached from the substratum, indicating a changed substrate adhesion of the VPA-treated cells. The data thus indicate that measurement of cellular area may serve as a simple in vitro test in the early pre-screening evaluation of teratogenicity of novel therapeutic agents.

Bishop JB, Witt KL, Sloane RA. **Genetic toxicities of human teratogens.** *Mutat Res* 1997;396(1-2):9-43.

BIOSIS COPYRIGHT: BIOL ABS. Birth defects cause a myriad of societal problems and place tremendous anguish on the affected individual and his or her family. Current estimates categorize about 3% of all newborn infants as having some form of birth defect or congenital anomaly. As more precise means of detecting subtle anomalies become available this estimate, no doubt, will increase. Even though birth defects have been observed in newborns throughout history, our knowledge about the causes and mechanisms through which these defects are manifested is limited. For example, it has been estimated that around 20% of all birth defects are due to gene mutations, 5-10% to chromosomal abnormalities, and another 5-10% to exposure to a known teratogenic agent or maternal factor (D.A. Beckman, R.L. Brent, Mechanisms of teratogenesis, *Ann. Rev. Pharmacol. Toxicol.* 24 (1984) 483-500; K. Nelson, L.B. Holmes, Malformations due to presumed spontaneous mutations in newborn infants, *N. Engl. J. Med.* 320 (1989) 19-23). Together, these percentages account for only 30-40%, leaving the

etiology of more than half of all human birth defects unexplained. It has been speculated that environmental factors account for no more than one-tenth of all congenital anomalies (D.A. Beckman, R. L. Brent, Mechanisms of teratogenesis, *Ann. Rev. Pharmacol. Toxicol.* 24 (1984) 483-500). Furthermore, since 'there is no evidence in humans that the exposure of an individual to any mutagen measurably increases the risk of congenital anomalies in his or her offspring' (J.F. Crow, C. Denniston, Mutation in human populations, *Adv. Human Genet.* 14 (1985) 59-121; J.M. Friedman, J.E. Polifka, Teratogenic Effects of Drugs: A Resource for Clinicians (TERIS), The John Hopkins University Press, Baltimore, 1994), the mutagenic activity of environmental agents and drugs as a factor in teratogenesis has been given very little attention. Epigenetic activity has also been given only limited consideration as a mechanism for teratogenesis. As new molecular methods are developed for assessing processes associated with teratogenesis, especially those with a genetic or an epigenetic basis, additional environmental factors may be identified. These are especially important because they are potentially preventable. This paper examines the relationships between chemicals identified as human teratogens (agents that cause birth defects) and their mutagenic activity as evaluated in one or more of the established short-term bioassays currently used to measure such damage. Those agents lacking mutagenic activity but with published evidence that they may otherwise alter the expressions or regulate interactions of the genetic material, i.e. exhibit epigenetic activity, have likewise been identified. The information used in making these comparisons comes from the published literature as well as from unpublished data of the U.S. National Toxicology Program (NTP).

Brinkworth MH, Anderson D, Hughes JA, Jackson LI, Yu TW, Nieschlag E. **Genetic effects of 1,3-butadiene on the mouse testis.** *Mutat Res* 1998;397(1):67-75.

BIOSIS COPYRIGHT: BIOL ABS. 1,3-Butadiene is a known male mouse germ-cell mutagen, to which humans may either be occupationally or environmentally exposed. Prolonged exposure to moderate or high doses in male mice can cause dominant lethal mutations and one report has indicated that 10 week inhalation administration of low doses can result in the production of malformed foetuses. The present study had dual purposes: (a) to attempt to clarify the suspected ability of sub-chronic (6 h/day, 5 days/wk, 10 weeks) low-dose exposure to 1,3-butadiene to induce heritable mutations in mouse male germ cells; (b) investigation of the relationships between testicular DNA damage, testicular DNA repair and foetal outcome. Adult male mice were exposed to low or moderate doses of 1,3-butadiene by inhalation sub-chronically or for a single 6 h period and either used for mating (sub-chronic exposure only) or for studies of DNA damage and repair. Litter size, dominant lethality and numbers of abnormal foetuses were determined the day preceding the normal day of parturition. Testicular DNA damage and repair were assessed by the Comet assay (for DNA damage) and the unscheduled DNA synthesis assay (for DNA repair). 1,3-Butadiene caused a statistically significant increase in dominant lethality at 125 ppm but not 12.5 ppm. No significant increase in DNA repair was found with either dose level or exposure period while only 6 h exposure to 125 ppm caused a small but significant increase in DNA damage as detected by the Comet assay. These effects demonstrate the reproductive genotoxicity of (125 ppm) 1,3-butadiene but do not confirm its ability to cause abnormalities in the offspring via the sperm. It is suggested that the relationship between 1,3-butadiene-induced DNA damage, DNA repair and heritable defects in the offspring may depend on the pattern of metabolites produced.

Chan DW, Yager TD. **Preparation and imaging of nuclear spreads from cells of the zebrafish**

embryo. Evidence for large degradation intermediates in apoptosis. *Chromosoma* 1998;107(1):39-60.

We describe a method for preparing nuclear spreads from cells of live, unfixed zebrafish embryos at the late-gastrula (approximately 8000 cell) stage of development. The method consists of a sequence of four steps: (1) a slow, gentle lysis, in low to moderate salt concentration, of cells and then nuclei, to release DNA-containing fibres; (2) spreading of the released fibres by a transverse fluid flow; (3) electrostatic, and possibly also covalent, attachment of the spread fibers to poly(L-lysine)-coated glass microscope slides; and (4) continued incubation to produce periodic cleavage of the DNA within the fibres, apparently through activation of endogenous nucleases. The nuclear spreads are imaged with epifluorescence, at a spatial resolution approaching the Rayleigh limit (approximately 230 nm for blue light). The epifluorescent signal is provided from Hoechst 33,258 bound specifically to the DNA, from a dye-coupled antibody conjugate bound specifically to histone H1 in the fibres, or from a DNA nick end-labelling assay. The spontaneous cleavage of DNA-containing fibres in step (4) of the above procedure can be blocked by the chelating agents EGTA and EDTA, by the caspase-2,3,7 inhibitor N-acetyl-Asp-Glu-Val-Asp-aldehyde, and by the caspase-1,4,5 inhibitors N-acetyl-Tyr-Val-Ala-Asp-aldehyde and N-acetyl-Tyr-Val-Ala-Asp-chloromethyl ketone. These data suggest that the spontaneous cleavage of fibres is catalysed by nucleases that become activated through a caspase-mediated mechanism. The involvement of caspase-dependent nucleases would suggest that an apoptosis pathway is activated in the spreads during their prolonged incubation. If bona fide apoptosis is induced in living zebrafish embryos by treatment with camptothecin (a topoisomerase I poison), and then nuclear spreads are prepared, we observe a similar fragmentation of the spread fibres. However, in this case the fragmentation is more rapid and complete. We hypothesize that, during the early phase of apoptosis, one or more endogenous nucleases are activated by a caspase-mediated mechanism. The nuclease(s) then specifically recognize and cleave a susceptible, periodically repeating feature of interphase chromatin.

Cooper MK, Porter JA, Young KE, Beachy PA. **Teratogen-mediated inhibition of target tissue response to Shh signaling [see comments].** *Science* 1998;280(5369):1603-7.

Veratrum alkaloids and distal inhibitors of cholesterol biosynthesis have been studied for more than 30 years as potent teratogens capable of inducing cyclopia and other birth defects. Here, it is shown that these compounds specifically block the Sonic hedgehog (Shh) signaling pathway. These teratogens did not prevent the sterol modification of Shh during autoprocessing but rather inhibited the response of target tissues to Shh, possibly acting through the sterol sensing domain within the Patched protein regulator of Shh response.

Dawson DA, Schultz TW, Hunter RS. **Developmental toxicity of carboxylic acids to *Xenopus* embryos: a quantitative structure-activity relationship and computer-automated structure evaluation.** *Teratog Carcinog Mutagen* 1996;16(2):109-24.

The developmental toxicity for each of 45 carboxylic acids was determined for *Xenopus* embryos. Acids tested included 12 unbranched, saturated aliphatics, 12 branched, saturated aliphatics, 12 unsaturated aliphatics, and 9 aromatics. Embryos were collected following hormone-induced breeding and exposed to at least eight concentrations of the acid, along with a control. For each concentration, 25 properly developing embryos were exposed to the acid solution for 96 h. Each acid was tested on at least three separate occasions and the data were pooled to calculate 96-h LC50 (lethality), 96-h EC50

(malformation), and DHI (developmental hazard index = 96-h LC50/96-h EC50) values. The endpoint data were subjected to quantitative structure-activity relationship (QSAR) analysis: and computer-automated structure evaluation (CASE). Variation in acid-induced lethality was effectively explained by partitioning and ionizability of the acids, while partitioning alone was somewhat effective in explaining variation for acid-induced malformation. The results indicated that developmental hazard of the acids to *Xenopus* embryos is primarily dependent on carbon-chain length, with acids containing five carbon atoms in the chain tending to be the most potent. Unsaturation reduced the hazard in comparison with the corresponding unbranched saturated acid. Developmental hazard was highest for 2-position branched compounds with a 5- or 6-C chain, but was reduced for 2-position branched acids with a 3- or 4-C chain. Hazard of the non-2 position branched acids was variable. Valproic (2-propylpentanoic) acid showed the highest developmental hazard with *Xenopus*, twice that of any other acid tested.

Finnell RH, Wlodarczyk BC, Craig JC, Piedrahita JA, Bennett GD. **Strain-dependent alterations in the expression of folate pathway genes following teratogenic exposure to valproic acid in a mouse model.** *Am J Med Genet* 1997 Jun 13;70(3):303-11.

The molecular basis for the well-established hierarchy of susceptibility to valproic acid-induced neural tube defects in inbred mouse strains was examined using *in situ* transcription and anti-sense RNA amplification methodologies with both univariate and multivariate analyses of the resulting gene expression data. The highly sensitive SWV strain demonstrated a significant reduction in the expression of the folate binding protein (FBP-1) following the teratogenic insult at gestational day 8:18, while the more resistant LM/Bc embryos were up-regulating this gene in response to valproic acid treatment. More importantly, at all 3 gestational timepoints spanning the period of murine neural tube closure examined in this study, the LM/Bc embryos had significantly higher MTHFR (5,10-methylenetetrahydrofolate reductase) gene expression levels compared to the SWV embryos. As this folate pathway enzyme is important in homocysteine and methionine metabolism, it suggests that the SWV embryos may be hypomethylated, and essential gene expression during critical periods of neural tube closure is compromised by the teratogenic exposure to valproic acid. This study represents the first evidence of a strain difference in transcriptional activity in response to a teratogenic exposure that might be causally related to the development of the teratogen-induced congenital malformations.

Fort DJ, Propst TL, Stover EL. **Evaluation of the developmental toxicity of 4-bromobenzene using frog embryo teratogenesis assay--*Xenopus*: possible mechanisms of action.** *Teratog Carcinog Mutagen* 1996;16(6):307-15.

Potential mechanisms of 4-bromobenzene-induced developmental toxicity were evaluated using frog embryo teratogenesis assay-*Xenopus* (FETAX). Early *X. laevis* embryos were exposed to 4-bromobenzene in two separate definitive concentration-response tests with and without an exogenous metabolic activation system (MAS) or selectively inhibited MAS. The MAS was treated with carbon monoxide (CO) to modulate P-450 activity, cyclohexene oxide (CHO) to modulate epoxide hydrolase activity, and diethyl maleate (DM) to modulate glutathione conjugation. Addition of the intact MAS, and particularly the CHO- and DM-inhibited MASs, dramatically increased the embryo lethal potential of 4-bromobenzene. Addition of the CO-inhibited MAS decreased the developmental toxicity of activated 4-bromobenzene to levels approximating that of the parent compound. Results from these studies suggested that a highly toxic arene oxide intermediate of 4-bromobenzene formed as the result of mixed

function oxidase (MFO)-mediated metabolism may play an important role in the development toxicity of 4-bromobenzene in vitro. Furthermore, both epoxide hydrolase and glutathione conjugation appeared to be responsible for activated 4-bromobenzene detoxification.

Fort DJ, Stover EL, Propst T, Hull MA, Bantle JA. **Evaluation of the developmental toxicity of theophylline, dimethyluric acid, and methylxanthine metabolites using *Xenopus*.** Drug Chem Toxicol 1996 Nov;19(4):267-78.

The developmental toxicities of theophylline and theophylline metabolites were evaluated using FETAX (Frog Embryo Teratogenesis assay - *Xenopus*). Young *X. laevis* embryos were exposed to theophylline, 1-methylxanthine, 3-methylxanthine, or 1, 3-dimethyluric acid 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. Addition of the MAS and CIM-MAS reduced the developmental toxicity of theophylline. Addition of the ELL- or CO-inhibited MAS did not reduce the developmental toxicity of theophylline. Addition of the intact MAS did not alter the developmental toxicity of 1-methyl- or 3-methylxanthine which were slightly more developmentally toxic on an equimolar basis than theophylline itself. 1, 3-dimethyluric acid was not developmentally toxic at maximum soluble concentrations in 1% (V/V) DMSO. Results from these studies suggested that P-450, specifically ELL-inhibited P-450 (aryl hydrocarbon hydroxylase) may have been responsible for detoxification of theophylline and that 1, 3 dimethyluric acid represented the primary detoxification metabolite of theophylline.

Fujimoto H, Tetsuo M, Tomonari M, Hamada T, Yakushiji M. **Growth-retardant and teratogenic effects of ketone bodies on early embryogenesis in rat whole embryo culture.** J Maternal-Fetal Invest 1997;7(2):92-8.

Objective: We investigated the effects of ketone bodies on early embryogenesis in the rat. We also assessed the influence of ketosis coexistence with hyperglycemia on fetal malformations, both of which occur in diabetic pregnancy that is poorly controlled. Methods: Nine-day-old rat embryos were cultured in vitro for 48 h by use of the whole embryo culture system of New. The effects of ketone bodies were studied by adding 4, 6, 8, and 16 mM of DL-beta-hydroxybutyrate into the culture medium. Hyperglycemic media were prepared with glucose concentrations of 300 or 600 mg/dL. Fetal growth was evaluated by direct measurement of crown-to-rump length and somatogenesis in embryos. Major and minor malformations were characterized by neural lesions and less severe extraneural lesions, respectively. Results: Growth retardation (less than or equal to 6 mM) and increased frequency of major (less than or equal to 6 mM) and minor malformations (less than or equal to 8 mM) were observed after 48-h exposure to DL-beta-hydroxybutyrate. The 4 mM DL-beta-hydroxybutyrate in the hyperglycemic medium (600 mg/dL glucose) led to a significant reduction in the number of somites and significant increase in the incidence of major and minor malformations, whereas there were fewer effects on the embryogenesis when each of them was used alone. Conclusion: Ketone bodies exerted an embryopathic effect on rat embryos during organogenesis and high glucose, and DL-beta-hydroxybutyrate acted synergistically to adverse effects on fetus.

Gabelova A,¹²⁶ Perin-Roussel O, Jounaidi Y, Perin F. **DNA adduct formation in primary mouse embryo**

cells induced by 7H-dibenzo[c,g]carbazole and its organ-specific carcinogenic derivatives. Environ Mol Mutagen 1997;30(1):56-64.

The nuclease P1 modification of the ³²P-postlabeling technique was used to study the biological activity of 7H-dibenzo[c,g]carbazole (DBC) and some of its derivatives, including N-methyldibenzo[c,g]carbazole (N-MeDBC), 5,9-dimethyldibenzo[c,g]carbazole (5,9-diMeDBC), 5,9,N-trimethyldibenzo[c,g]carbazole (5,9,N-triMeDBC), 6-methoxydibenzo[c,g]carbazole (6-MeODBC), N-acetyldibenzo[c,g]carbazole (N-AcDBC), N-hydroxymethyldibenzo[c,g]carbazole (N-HMeDBC) in primary mouse embryo cells. A very good correlation was found between carcinogenic specificity in vivo of these N-heterocyclic aromatic hydrocarbons and their DNA-adduction in vitro. Primary mouse embryo cells were able to metabolize and detect tissue-specific sarcomagens N-MeDBC and 6-MeODBC as well as derivatives with both sarcomagenic and hepatocarcinogenic activity, DBC, N-AcDBC, and N-HMeDBC. The strong specific hepatocarcinogen 5,9-diMeDBC in vivo, did not induce any DNA-adducts in the embryo cells, which suggests that the enzymatic composition of the target tissue probably is the determining factor in the organ specificity of this derivative. 5,9,N-triMeDBC, derivative without any carcinogenic activity in vivo, did not induce any DNA-adducts in primary mouse embryo cells. Pretreatment of cells with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) apparently stimulated DNA-adduct formation in the cells exposed to DBC, 6-MeODBC, and N-MeDBC. No or a very slight effect of TCDD on DNA-adduct formation was found in cells exposed to N-HMeDBC and N-AcDBC. Preliminary results have shown that TCDD slightly induced cytochrome P4501A1-linked ethoxyresorufin O-deethylase (EROD) activity in primary mouse embryo cells. These data suggest the role of cytochrome P4501A1 in the metabolism of DBC derivatives with sarcomagenic activity.

Ghanooni M, Mattison DR, Zhang YP, Macina OT, Rosenkranz HS, Klopman G. **Structural determinants associated with risk of human developmental toxicity.** Am J Obstet Gynecol 1997 Apr;176(4):799-805; Discussion 805-6.

OBJECTIVES: Identifying drugs or chemicals that represent hazards to human development is a continuous challenge. Of the approximately 60,000 chemicals in commercial use only 5% have been evaluated for developmental toxicity. Identification of inexpensive, rapid, validated techniques to demonstrate chemical hazards for the human embryo or fetus is the objective of this research. **STUDY DESIGN:** This research explored identification of structure activity predictors associated with human developmental toxicity by means of MULTICASE (multiple computer-automated structure evaluation), an algorithm that evaluates associations between chemicals and their constituent fragments and a biologic response. This algorithm allows identification of chemicals (and specific substructures) that may be human developmental toxicants. Developmental toxicity data were compiled from two sources (the Teratogen Information System and Food and Drug Administration guidelines) and analyzed to identify structural determinants (biophores) associated with human developmental toxicity. **RESULTS:** This analysis identified 17 biophores associated with human developmental toxicity. Testing the biophores against the learning set demonstrated 99% concordance, 100% sensitivity, and 98% specificity. Cross-validation studies were conducted, in which the original database was randomly separated into five learning and test sets; these demonstrated a mean concordance of 73%, with a mean sensitivity of 63% and a mean specificity of 79%. **CONCLUSIONS:** The MULTICASE structure-activity model is useful for identifying potential human developmental toxicants, as well as serving as a starting point for mechanistic investigations.

Gray LEJ, Ostby J, Wolf C, Lambright C, Kelce W. **The value of mechanistic studies in laboratory animals for the prediction of reproductive effects in wildlife: endocrine effects on mammalian sexual differentiation.** Environ Toxicol Chem 1998;17(1):109-18.

BIOSIS COPYRIGHT: BIOL ABS. Wildlife populations from contaminated ecosystems display a variety of reproductive alterations, including cryptorchidism in the Florida panther, small baculum in young male otters, small penises in alligators, sex reversal in fish, and altered social behavior in birds. The formation of biologically plausible hypotheses regarding disruption of reproduction in wildlife can be facilitated by mechanistic studies on laboratory animals. To this end, we are investigating the in vivo and in vitro effects of endocrine-disrupting toxicants in rodents. In vitro studies have used receptor binding and transfected cell assays to confirm the suspected mechanism of action, whereas in vivo rodent studies examine altered sexual differentiation. Antiandrogenic pesticides compete with the natural ligands for both rat and human androgen receptors, block androgen-induced gene expression in vitro and in vivo, delay puberty, reduce sex accessory gland size, and alter male rat sex differentiation. In contrast, xenoestrogens affect female central nervous system sex differentiation and fecundity without producing malformations or infertility in male offspring. Prenatal administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or the TCDD-like polychlorinated biphenyls produce yet another profile of effects in the offspring, reducing numbers of ejaculated sperm in male progeny and inducing urogenital malformations in females. Although phthalates are reported to be estrogenic in vitro, in vivo exposure causes developmental alterations that more closely resemble antiandrogenic activity. The mammalian data indicate that exposure to endocrine-disrupting chemicals produces effects that are pathognomonic for mechanisms by which they act. Mechanistic information derived from mammalian studies can enhance our ability to predict toxicant effects on reproduction in fish and wildlife.

Guerri C. **Teratogenic effects of alcohol: current status of animal research and in vitro models.** Arch Toxicol Suppl 1996;18:71-80

Hoar RM. **Developmental toxicity: extrapolation across species.** J Am Coll Toxicol 1995;14(1):11-20.

There is a pervasive lack of understanding about the definition of a developmental toxicant and the experimental design necessary to differentiate between maternal toxicants and those uniquely, i.e., selectively, hazardous to the conceptus. Thus, the acceptance of the adult-to-developmental (A/D) ratio and its ability to identify those compounds uniquely hazardous to the conceptus has been compromised, as has the extrapolation of hazard across species. The failure to establish a shared understanding is discussed, together with the role of an in vitro screen for determining the A/D ratio. The addition of an environmental exposure factor permitting the extrapolation of hazard assessment across species and the estimation of potential human developmental toxicity is detailed.

Honda A, Tint GS, Shefer S, Batta AK, Honda M, Salen G. **Effect of YM 9429, a potent teratogen, on cholesterol biosynthesis in cultured cells and rat liver microsomes.** Steroids 1996 Sep;61(9):544-8. YM 9429 (cis-1-[4-(p-menthan-8-yloxy)phenyl]piperidine) is a hypolipidemic agent with a potent and specific teratogenicity, inducing cleft palate and skeletal variations in rats. Since cleft palate is generally observed in the Smith-Lemli-Opitz syndrome, a common syndrome of multiple congenital anomalies

caused by reduced activity of 7-dehydrocholesterol delta 7-reductase (3 beta-hydroxysteroid delta 7-reductase), the final enzyme in the cholesterol biosynthetic pathway, YM 9429 was suspected of being an inhibitor of this enzyme. To prove this hypothesis, YM 9429 was added to cultured human skin fibroblasts and to cultured Morris hepatoma cells and incubated with [5-3H]mevalonolactone. After 24 h, radiolabeled 7-dehydrocholesterol accumulated in the cells, whereas the formation of radiolabeled cholesterol was markedly reduced. The results indicate that YM 9429 inhibits the conversion of 7-dehydrocholesterol to cholesterol catalyzed by the microsomal enzyme 7-dehydrocholesterol delta 7-reductase. In rat liver microsomes, the mode of inhibition was found to be noncompetitive, with a K_i of 40 microM. These results suggest that YM 9429 induced developmental abnormalities in rats by the same mechanism as the Smith-Lemli-Opitz syndrome. This compound might be useful for studying the pathogenesis of anomalies in animal models of the Smith-Lemli-Opitz syndrome.

Jacobson JL, Jacobson SW. **Teratogen update: polychlorinated biphenyls.** *Teratology* 1997 May;55(5):338-47.

Jiang H, Soprano DR, Li SW, Soprano KJ, Penner JD, Gyda M 3rd, Kochhar DM. **Modulation of limb bud chondrogenesis by retinoic acid and retinoic acid receptors.** *Int J Dev Biol* 1995 Aug;39(4):617-27.

An excess of retinoic acid (RA) in the mouse embryo in utero produces hypochondrogenesis and severe limb bone deformities. Since one of the RA receptors--RAR-beta 2, is specifically induced in the limb bud cells upon treatment of embryos with teratogenic doses of RA, we investigated if this receptor played a role in teratogenesis by regulating the process of chondrogenesis. In micromass cultures of mouse limb bud mesenchymal cells, we found that a downregulation of RAR-beta 2 as well as several other RAR isoforms by supplementation of the culture medium with specific oligodeoxynucleotides stimulated chondrogenesis: cartilage nodule number, sulfated proteoglycans, and synthesis of collagen type IIB were all enhanced in a dose-dependent manner. However, only the antisense RAR-beta 2 probe efficiently prevented the strong inhibitory effects of exogenous RA on chondrogenesis in these cells. The data suggest that the RAR-RA complexes play a role in position-dependent patterning of the limb skeleton in normal development and that, in particular, RAR-beta 2 serves to prevent the mesenchymal cells from expressing their chondrogenic bias. Our results further strengthen the argument that RA-dependent elevation in RAR-beta 2 levels plays a unique role in RA-induced teratogenesis.

Kapron CM, Trasler DG. **Genetic determinants of teratogen-induced abnormal development in mouse and rat embryos in vitro.** *Int J Dev Biol* 1997 Apr;41(2):337-44.

The response of an embryo to a teratogenic treatment is often critically dependent on its genetic makeup. However, in conventional in vivo studies of gene-teratogen interactions it may be difficult to distinguish between the effects of genes that are carried by the embryo and those that are carried by the mother. It is likewise not easy to determine whether an observed interaction is between a particular gene and the parent compound administered, or whether it is with a metabolite that has been generated by the maternal system. The use of whole rodent embryo culture offers certain advantages in the study of gene-teratogen interactions. Not only can the effects of metabolism and the maternal genotype be more carefully controlled, but the stage of development at which embryos of different genotypes are exposed can be matched. Rodent whole embryo culture has been used to a limited extent to study interactions

between single gene mutations and teratogenic treatments, variations in responses of different strains to teratogens, as well as species differences in response to teratogens. These studies point to the need to precisely control the stage of development at the time of treatment in order to be able to make valid comparisons. But, even more important, they highlight the versatility of the whole embryo culture technique, and underscore the need for its wider use in evaluating the relative contribution of genes and environment to abnormal embryonic development.

Kavlock RJ. Recent advances in mathematical modeling of developmental abnormalities using mechanistic information. *Reprod Toxicol* 1997 Mar-Jun;11(2-3):423-34.

During the last several years, significant changes in the risk assessment process for developmental toxicity of environmental contaminants have begun to emerge. The first of these changes is the development and beginning use of statistically based dose-response models [the benchmark dose (BMD) approach] that better utilize data derived from existing testing approaches. Accompanying this change is the greater emphasis placed on understanding and using mechanistic information to yield more accurate, reliable, and less uncertain risk assessments. The next stage in the evolution of risk assessment will be the use of biologically based dose-response (BBDR) models that begin to build into the statistically based models factors related to the underlying kinetic, biochemical, and/or physiologic processes perturbed by a toxicant. Such models are now emerging from several research laboratories. The introduction of quantitative models and the incorporation of biologic information into them has pointed to the need for even more sophisticated modifications for which we offer the term embryologically based dose-response (EBDR) models. Because these models would be based upon the understanding of normal morphogenesis, they represent a quantum leap in our thinking, but their complexity presents daunting challenges both to the developmental biologist and the developmental toxicologist. Implementation of these models will require extensive communication between developmental toxicologists, molecular embryologists, and biomathematicians. The remarkable progress in the understanding of mammalian embryonic development at the molecular level that has occurred over the last decade combined with advances in computing power and computational models should eventually enable these as yet hypothetical models to be brought into use.

Kelce WR, Wilson EM. Environmental antiandrogens: developmental effects, molecular mechanisms, and clinical implications. *J Mol Med* 1997 Mar;75(3):198-207.

Industrial chemicals and environmental pollutants can disrupt reproductive development in wildlife and humans by mimicking or inhibiting the action of the gonadal steroid hormones, estradiol and testosterone. The toxicity of these so-called environmental endocrine disruptors is especially insidious during sex differentiation and development due to the crucial role of gonadal steroid hormones in regulating these processes. This review describes the mechanism of toxicity and clinical implications of a new class of environmental chemicals that inhibit androgen-mediated sex development. For several of these chemicals, including the agricultural fungicide vinclozolin and the ubiquitous and persistent 1,1,1-trichloro-2,2-bis (p-chlorophenyl)ethane metabolite, 1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene, the molecular mechanism of action and the adverse developmental effects on male sex differentiation have been elucidated and are used as examples. Environmental chemicals with antiandrogenic activity offer profound implications with regard to recent clinical observations that suggest an increasing incidence of human male genital tract malformations, male infertility, and female breast cancer. Finally, in light of

increasing concern over the potential endocrine disrupting effects of environmental pollutants, an in vitro/in vivo investigational strategy is presented which has proved useful in identifying chemicals with antiandrogen activity and their mechanism of action.

Kimmel CA. Developmental toxicity risk assessment: consensus building, hypothesis formulation, and focused research. Drug Metab Rev 1996 Feb-May;28(1-2):85-103.

Risk assessment for developmental toxicity has become more defined over the last decade and the most recent EPA guidelines for developmental toxicity risk assessment were published in 1991. Development of approaches for risk assessment in this area have relied on building of consensus opinion among experts about the interpretation of developmental toxicity data. These discussions have aided in strengthening the scientific basis for risk assessment for developmental toxicity, including the default assumptions that must be used when more complete information on mechanisms of action and pharmacokinetics are unavailable. Such discussions continue on both a national and international basis. The EPA risk assessment guidelines outlined several major areas of research needed to strengthen risk assessment for developmental toxicity and led to the formulation of hypotheses to be tested in focused research projects. Several major research efforts have focused on dose-related mechanisms and biologically based modeling of specific agents, physiologically based pharmacokinetic models of pregnancy that can be scaled across species, and the influence of dose and duration of exposure on developmental outcomes, as well as the delineation of specific biomarkers of adverse developmental effects. Although such research initiatives will require a long-term effort, it is important that attention be focused now on those approaches that can improve the risk assessment process to avoid continued reliance on default approaches that have been used for the past 30 years or more. The impact of the EPA Guidelines for Developmental Toxicity Risk Assessment has been widespread. One of the main purposes for developing risk assessment guidelines by EPA was to communicate procedures to risk assessors inside the agency in an effort to promote consistency. In addition, the guidelines were to communicate to those outside the agency in private industry and other regulatory agencies how EPA would evaluate and interpret data. The developmental toxicity guidelines have standardized terminology used in developmental toxicity risk assessments. They also have influenced the revision of testing guidelines and the writing of new guidelines, such as the developmental neurotoxicity testing guidelines. More extensive use of developmental toxicity data within the RfD/RfC process was influenced by the guidelines, as well. More recently, the risk assessment guidelines have had a major impact on the harmonization of international testing and risk assessment guidelines through cooperative efforts with the World Health Organization under the auspices of the International Program on Chemical Safety (IPCS) and the Organization for Economic Cooperation and Development (OECD). We foresee these types of interagency and international cooperative efforts continuing as we attempt to make more efficient use of the limited resources available for testing, risk assessment, and research to better understand and prevent developmental toxicity.

Kochhar DM, Jiang H, Penner JD, Beard RL, Chandraratnaas. Differential teratogenic response of mouse embryos to receptor selective analogs of retinoic acid. Chem Biol Interact 1996 Mar 8;100(1):1-12.

Early events that initiate teratogenesis by Accutane or other retinoids in mammalian embryos remain unknown. It would be helpful for mechanistic considerations to know whether or not retinoids act

through retinoid receptor-dependent pathways, and if they do, which of the two families of receptors (retinoic acid receptors - RAR α , β , γ or retinoid X receptors - RXR α , β , γ) are more likely involved. We previously used an in vitro bioassay to demonstrate that those retinoid analogs with binding affinity and transactivational activity limited only to the RXRs have a low potential as teratogens. Here, we have extended the study to examine teratogenicity, in pregnant mice, of a number of synthetic retinoids with varying degrees of receptor selectivity. The ability of each compound to induce fetal limb and craniofacial defects after a single exposure on day 11 of gestation was assessed and compared to that of all-trans retinoic acid (RA). The highest dose selected was 100 mg/kg maternal body weight since such a regimen of all-trans RA affects virtually every exposed embryo without any indication of maternal toxicity. We found that although all RAR agonists were strong teratogens, their potencies varied over a wide magnitude. The teratogenic potencies and receptor transactivation profiles of RAR agonists were not directly correlated since compounds with similar receptor activities presented major differences in potencies. Three compounds were exclusively RXR agonists, and these were not teratogenic under our experimental conditions. Two additional compounds which turned out to be non-teratogenic were distinguished by the fact that they activated neither RARs nor RXRs. These data indicate that although RAR-dependent mechanisms are likely involved in retinoid-induced teratogenesis, there are additional factors which determine teratogenic potency. The absence of teratogenic response in the case of RXR agonists suggests that risk-benefit analyses of such receptor-selective compounds may be fruitful in further studies.

Kolf-Clauw M, Chevy F, Wolf C, Siliart B, Citadelle D, Roux C. **Inhibition of 7-dehydrocholesterol reductase by the teratogen AY9944: a rat model for Smith-Lemli-Opitz syndrome.** *Teratology* 1996 Sep;54(3):115-25.

Our aim is to verify the validity of a rat model proposed for Smith-Lemli-Opitz (SLO) syndrome, a developmental disorder characterized by a defect in 7-dehydrocholesterol-delta 7 (7DHC)-reductase and by facial dysmorphism close to the holoprosencephaly caused by the teratogen AY9944. We investigated the sterol profile in rats treated with AY9944 blocking 7DHC-reductase. AY9944 was given orally to rats on gestation day 3 (D3). The sera were sampled for kinetic data on D3, D6, D9, D12, and D21. Cholesterol was measured in parallel by the routine enzymatic method and by the gas chromatography/mass spectrometry (GC-MS) procedure used in SLO diagnosis. In addition to sterols, we dosed steroid hormones punctually on D4 and on D10, and examined D21 fetuses in other animals. The enzymatic method was not specific for cholesterol, and measured 70% pure 7DHC added to a normal serum. On D21, 77% live fetuses showed pituitary agenesis. Cholesterol was rapidly reduced by more than 50% on D6 involving an accumulation of 7DHC, 8DHC, and trienols, as identified in SLO-affected children. The most abundant 7DHC reached a maximum from D9 to D12, equaling cholesterol on D9 (11 mg/dl). On D10, the magnitudes of hypocholesterolemia and of 7DHC accumulation were found to be dose-dependent. Progesterone was reduced as early as 24 hr after treatment and dropped to 40% of the levels in the controls on D10, correlating to the decrease in cholesterolemia. This rat model reproduces the same biochemical perturbations as seen in SLO, strongly suggesting that aberrant sterols (7DHC, 8DHC, or nortrienol) may contribute to the developmental defects.

Leroux BG, Leisenring WM, Moolgavkar SH, Faustman EM. **A biologically-based dose-response model for developmental toxicology.** *Risk Anal* 1996 Aug;16(4):449-58.

The methods currently used to evaluate the risk of developmental defects in humans from exposure to potential toxic agents do not reflect biological processes in extrapolating estimated risks to low doses and from test species to humans. We develop a mathematical model to describe aspects of the dynamic process of organogenesis, based on branching process models of cell kinetics. The biological information that can be incorporated into the model includes timing and rates of dynamic cell processes such as differentiation, migration, growth, and replication. The dose-response models produced can explain patterns of malformation rates as a function of both dose and time of exposure, resulting in improvements in risk assessment and understanding of the underlying mechanistic processes. To illustrate the use of the model, we apply it to the prediction of the effects of methylmercury on brain development in rats.

Little J. **Are occupational carcinogens teratogenic?** *Reprod Toxicol* 1993;:3-43.

A variety of congenital anomalies have been associated with neoplasms in humans. In particular, acute lymphocytic leukaemia in childhood is associated with Bloom's syndrome, Fanconi's anaemia and Down's syndrome, lymphomas are associated with ataxia-telangiectasia, Wiskott-Aldrich syndrome and thymic aplasia, and Wilms' tumor has been associated with a variety of congenital anomalies including aniridia, hemihypertrophy, and Beckwith-Wiedemann syndrome. These associations relate primarily to cancer in childhood and some have a clear genetic basis. However, carcinogens are inferred to interfere with cell function and such interference would be likely to affect developmental processes. Data on experimental carcinogenesis show that many agents known to be carcinogenic postnatally in animals are teratogenic to the fetus or embryo. For example, Di Paolo and Kotin listed 26 chemical agents that had been tested for both carcinogenic and teratogenic activity in animals. Of the 20 listed as carcinogenic, 19 had been shown to be teratogenic. Carcinogens have been identified from the IARC Monographs. Exposure to many of the carcinogens identified in the Monographs occurs in an occupational context and, therefore, the aim of this chapter is to assess the evidence as to the teratogenicity in humans of occupational exposures considered to be carcinogenic. First, the framework used to evaluate evidence as to the carcinogenicity of the occupational exposures will be described. Next, the sources of information on teratogenicity will be specified. Evidence regarding the teratogenicity of occupational exposures for which data are available on humans will then be considered. This will be followed by consideration of exposures for which data are available only from experimental animals. Finally, mechanisms whereby exposures may produce both teratogenic and carcinogenic effects will be discussed.

Luecke RH, Wosilait WD, Pearce BA, Young JF. **A computer model and program for xenobiotic disposition during pregnancy.** *Comput Methods Programs Biomed* 1997 Jul;53(3):201-24.

A physiologically based pharmacokinetic computer model and program have been developed that depict internal disposition of chemicals during pregnancy in the mother and embryo/fetus. The model is based on human physiology but has been extended to simulate laboratory animal data. The model represents the distribution, metabolism, and elimination of two chemicals in both the maternal and embryo/fetal systems; the program handles the two chemicals completely independently or interactively with the two chemicals sharing routes of metabolism and/or elimination. The FORTRAN program computes the concentration of the two chemicals in 26 organs/tissues in the pregnant mother and 15 organs/tissues in the embryo/fetus using a 486DX4 or Pentium PC. Adjustments for embryo/fetal organ and tissue

volumes as a function of developmental age are made utilizing the Gompertz growth equation for the developing embryo/fetus and allometric relationships for the developing organs. Various changes in the maternal compartments which could affect the distribution of a xenobiotic during pregnancy are also included in the model. Input files require estimates of binding coefficients, first- and/or second-order metabolism constants, level of interaction between the two chemicals, and dosing information. Different possible routes of administration are included (e.g., i.v., infusion, oral, dermal, and inhalation, as well as repeated doses or exposures). Regression analysis can be conducted on any combination of these various parameters to fit actual data. Output concentration-time curves are available simultaneously from all 82 differential equations. An illustrative example compares observed data with simulations for imipramine and its demethylated metabolite, desipramine, in both the maternal rat and her fetuses. Methyl mercury data for the non-pregnant and pregnant rat also are compared with human data. Based on parameters determined from analysis of rat data, the model is readjusted for human physiology and predicts human maternal and fetal tissue concentrations as a function of time.

Lyn SD. **Whole-mount in situ hybridization of mouse embryos exposed to teratogenic levels of retinoic acid.** *Meth Mol Biol* 1998;89:67-79.

BIOSIS COPYRIGHT: BIOL ABS. RRM BOOK CHAPTER MOUSE EMBRYO MOLECULAR GENETICS METHODOLOGY WHOLE-MOUNT IN-SITU HYBRIDIZATION RETINOIC ACID TERATOGEN GENE EXPRESSION ANALYSIS MRNA DETECTION MESSENGER RNA DEVELOPMENT ANALYTICAL METHOD.

Maar TE, Ellerbeck U, Bock E, Nau H, Schousboe A, Berezin V. **Prediction of teratogenic potency of valproate analogues using cerebellar aggregation cultures.** *Toxicology* 1997 Jan 15;116(1-3):159-68.

The aim of this study was to develop a novel in vitro system suitable for preclinical testing for developmental toxicity of drugs. An assay system consisting of primary cultures of dissociated cerebella from 6-day-old mice was chosen, since it allowed quantification of neuronal aggregation and fasciculated neurites. A human teratogen, the antiepileptic drug valproic acid (VPA), as well as its structural analogues, (+/-)-4-en-VPA and E-2-en-VPA, with varying teratogenic activities, were tested and found to affect aggregation and fiber formation of cerebellar neurons. Based on a dose-response study, the concentrations of compounds causing 50% inhibition (IC₅₀) of formation of thick and thin fibers were determined. The lowest IC₅₀ values were found for VPA (52 +/- 7 and 86 +/- 11 microM for thick and thin fibers, respectively), which in vivo caused the highest rate of exencephaly among the three compounds tested, (+/-)-4-en-VPA exhibited intermediate values (150 +/- 30 and 300 +/- 40 microM), whereas the highest IC₅₀ values were found for E-2-en-VPA (260 +/- 42 and 430 +/- 40 microM). The latter compound does not induce neural tube defects, but has been shown to have neurobehavioral effects in prenatally exposed animals. Subsequently, the purified S- and R-enantiomers of 4-yn-VPA (teratogenic and non-teratogenic, respectively) were tested for their effects on aggregation and fiber formation of the cerebellar neurons. Treatment with S-4-yn-VPA resulted in pronounced changes in numbers of aggregates and fasciculated processes compared to the cultures treated with R-4-yn-VPA, indicating that the intrinsic stereoselective potency of the enantiomers may be correlated to the difference in their effects on cerebellar neurons in vitro. Thus, the teratogenic potency of VPA and its analogues correlated with their effects on aggregation of neural cells and formation of fasciculated neurites in primary cultures of dissociated cerebella, indicating that the in vitro assay system employed

may be used as a pre-screening test for prediction of teratogenic potency of drugs.

Miller RK, Hendrickx AG, Mills JL, Hummler H, Weigand UW. **Periconceptional vitamin A use: how much is teratogenic?** *Reprod Toxicol* 1998;12(1):75-88.

BIOSIS COPYRIGHT: BIOL ABS. The objective of the review is to determine whether preformed vitamin A (retinol and retinyl esters) is teratogenic at dosages commonly used by women living in industrialized countries. Published human and animal data and research developed by the authors are reviewed. It is well known that vitamin A is essential for normal reproduction and development. Although doses of 10,000 IU/d or less of preformed vitamin A (retinyl esters and retinol) are considered safe, doses >10,000 IU/d as supplements have been reported to cause malformations in a single epidemiologic study. Nonhuman primate data show no teratogenicity at doses of 30,000 IU/d. Daily periconceptional exposures greater than 25,000 IU/d of preformed vitamin A have not been sufficiently studied to establish specific risk. Because no study reports adverse effects of 10,000 IU/d preformed vitamin A supplements and this dose is more than the Recommended Dietary Allowance for pregnant women (2670 IU or 800 RE/d), we recommend that women living in industrialized countries or who otherwise have nutritionally adequate diets may not need to ingest more than the Recommended Dietary Allowance of preformed vitamin A as supplements. If periconceptional vitamin A exposures to levels up to 30,000 IU/d (9,000 mug RE/d) do occur unintentionally, multiple animal studies do support only very low risk. Human epidemiologic studies do not establish at what level vitamin A becomes teratogenic; however, pharmacokinetic data presented in this paper indicate that blood levels of retinoids from women taking 30,000 IU/d of preformed vitamin A are not greater than retinoid blood levels in pregnant women during the first trimester who delivered healthy babies. Interestingly, neither teratogenicity nor vitamin A toxicity has been observed in multiple species exposed to high doses of beta-carotene.

Minucci S, Leid M, Toyama R, Saint-Jeannet JP, Peterson VJ, Horn V, Ishmael JE, Bhattacharyya N, Dey A, Dawid IB, et al. **Retinoid X receptor (RXR) within the RXR-retinoic acid receptor heterodimer binds its ligand and enhances retinoid-dependent gene expression.** *Mol Cell Biol* 1997 Feb;17(2):644-55.

Retinoic acid receptor (RAR) and retinoid X receptor (RXR) form heterodimers and regulate retinoid-mediated gene expression. We studied binding of RXR- and RAR-selective ligands to the RXR-RAR heterodimer and subsequent transcription. In limited proteolysis analyses, both RXR and RAR in the heterodimer bound their respective ligands and underwent a conformational change in the presence of a retinoic acid-responsive element. In reporter analyses, the RAR ligand (but not the RXR ligand), when added singly, activated transcription, but coaddition of the two ligands led to synergistic activation of transcription. This activation required the AF-2 domain of both RXR and RAR. Genomic footprinting analysis was performed with P19 embryonal carcinoma cells, in which transcription of the RARbeta gene is induced upon retinoid addition. Paralleling the reporter activation data, only the RAR ligand induced in vivo occupancy of the RARbeta2 promoter when added singly. However, at suboptimal concentrations of RAR ligand, coaddition of the RXR ligand increased the stability of promoter occupancy. Thus, liganded RXR and RAR both participate in transcription. Finally, when these ligands were tested for teratogenic effects on zebra fish and *Xenopus* embryos, we found that coadministration of the RXR and RAR ligands caused more severe abnormalities in these embryos than either ligand alone, providing biological support for the synergistic action of the two ligands.

Mirkes PE. **Prospects for the development of validated screening tests that measure developmental toxicity potential: view of one skeptic.** *Teratology* 1996 Jun;53(6):334-8.

Humans are exposed to a variety of potential developmental toxicants. This fact, combined with the knowledge that human development can be disrupted by environmental agents, has led to the development of methods designed to identify potential developmental toxicants. Currently, the principal method used to screen drugs and chemicals that are potential human developmental toxicants is the segment II study (i.e., a study in which prospective drugs and chemicals are tested in pregnant animals). Because of the cost and time involved in such studies and the pressure to reduce the number of animals used in such testing, alternative methods for developmental toxicity testing have been sought. This has resulted in a number of in vitro tests whose aim is to screen large numbers of agents quickly and inexpensively. Although numerous in vitro tests of developmental toxicity have been developed during the last 15 years, no one system or combination of tests have been validated for the purpose intended. Nonetheless, two systems--the limb bud/CNS micromass, and the chick embryo neural retina cell culture (CERC)--continue to be advanced as viable in vitro developmental toxicology tests. The purpose of this commentary is to evaluate the prospects for the development of an in vitro test system(s) that can screen the universe of drugs and chemicals and reliably identify those that require further study and those that do not. The conclusion of this investigator is that the prospects for validating such in vitro tests are not promising. This conclusion is based primarily on the lack of basic knowledge regarding the relevance of end points assayed in the micromass and CERC test systems to those end points known or thought to be critical for normal development.

Mizell M, Romig ES. **The aquatic vertebrate embryo as a sentinel for toxins: zebrafish embryo dechoriation and perivitelline space microinjection.** *Int J Dev Biol* 1997 Apr;41(2):411-23.

Pollution of aquatic ecosystems poses a serious threat to aquatic organisms and ultimately the entire ecosystem. Understanding how a toxin affects embryonic development is key to determining the risk a pollutant represents to the environment. Extraembryonic membranes, such as the chorion of fish eggs, provide a protective barrier between the embryo and the environment. Although the fish chorion excludes many chemical pollutants, some noxious agents can still gain access to the aquatic embryo. Therefore a monitoring system that tests the effects directly upon the embryo must be established. Although exposure to a single toxin in the laboratory can determine the concentration at which a pollutant becomes a health or environmental hazard, embryos and adults in nature are not merely affected by a single chemical, but are exposed to mixtures of different pollutants. Zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*) embryos were employed for the rapid observation of the effects of single chemicals and chemical mixtures on development. Using dechoriation and a perivitelline space microinjection system, the embryos were effective sentinels for low concentrations of aquatic pollutants. The developmental effects of small quantities of toxins were observed. Embryos treated during the late gastrula stage of development with hexachlorobenzene (HCB); 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD); toluene; benzene; or mixtures of these chemicals developed cardiovascular abnormalities. The zebrafish dechoriation exposure technique, Micro Intrachorionic Zebrafish Embryo Live Laboratory test, was especially effective in testing the pollutant mixtures. Combinations of both TCDD and benzene (as well as the toluene and benzene combinations) were tested and the mixtures acted synergistically; the combinations were more toxic than either chemical by itself. Hexachlorobenzene- and TCDD-treated

embryos tested positively for expression of cytochrome P450 1A indicating that the cytochrome metabolic pathways were already functional in these early embryos, and suggested that a product of the cytochrome system may be involved in HCB and TCDD pollution associated cardiovascular defects.

Pafkova H, Jerabek J, Tejnorova I, Bednar V. **Developmental effects of magnetic field (50 Hz) in combination with ionizing radiation and chemical teratogens.** *Toxicol Lett* 1996 Nov;88(1-3):313-6. The influence of a 50 Hz magnetic field (MF) on avian and mammalian embryogenesis, the MF level and vector, as well as the effect of exposure to MF (50 Hz, 10 mT) in combination with X-rays has been recently reported [2,3]. No significant alterations of chick or rat embryogenesis were found after repeated exposures to 50 Hz MF at 10 mT or 6 microT or with different vectors. However, X-ray chick embryotoxicity was significantly affected by repeated exposures of developing organisms to MF. A strong dependence of effect on the type of interaction was revealed. A decrease of X-ray induced teratogenicity was observed when MF preceded X-ray exposure (indirect interaction), while MF exposure applied immediately after X-ray radiation (direct interaction) non-significantly potentiated adverse developmental effects of ionizing radiation. This study deals with the effects of MF in combination with insulin or tetracycline. Exposure of chick embryos to MF influenced the sensitivity of embryonic morphogenetic systems to the subsequently administered chemical teratogens, insulin and/or tetracycline. A protective effect of MF was detected similarly as in the case of indirect interaction with ionizing radiation.

Peterka M, Peterkova R, Likovsky Z. **Teratogenic and lethal effects of long-term hyperthermia and hypothermia in the chick embryo.** *Reprod Toxicol* 1996 Jul-Aug;10(4):327-32.

The teratogenic effect of maternal hyperthermia is well known in laboratory animals and is presumed to exist also in humans. The aim of our study was to describe the embryotoxic effect of long-term higher and lower incubation temperatures on the chick embryo. Chick embryos were incubated within days 1 to 9 at 12 different incubation temperatures ranging from 31 to 42 degrees C. On the basis of our results, we estimated that there are three upper and lower critical thresholds of the incubation temperature: the first thresholds are 31 and 42 degrees C, at which all embryos died; the second thresholds are 32 and 41 degrees C, at which all living embryos were malformed; the third thresholds are 33 and 40 degrees C, at which some of the living embryos were without structural malformations, but their weight was shifted down and up with lower and higher temperature, respectively. The incubation temperature of 37 to 38 degrees C was optimal. Typical malformations detected on day 9 of incubation were microphthalmia, gastroschisis, caudal regression syndrome, and hyperlordosis, all of which occurred in dead embryos several times more frequently than in living embryos. CNS malformations were only sporadically present on day 9, as most of specimens bearing CNS defects died during the first days of incubation.

Piersma AH, Bechter R, Krafft N, Schmid BP, Stadler J, Verhoef A, Verseil C, Zijlstra J. **An interlaboratory evaluation of five pairs of teratogens and non-teratogens in post-implantation rat embryo culture.** *Alternatives Lab Anim* 1996;24(2):201-9.

The usefulness of the post-implantation rat embryo culture method in screening xenobiotic compounds for developmental toxicity was validated in four laboratories with five pairs of compounds. This approach was chosen to provide information on the interlaboratory reproducibility of the results and to compare the effects of chemical analogues in embryo culture. By testing analogous compounds which

are known to have different embryotoxic potencies in vivo, the discriminating power of the embryo culture method for the compound classes under study could be optimally assessed. The classes selected for testing were triazole antifungals, phthalic ester metabolites, substituted pyridines, sulphonamides and methylated xanthines. In summary, it was possible to distinguish between the compounds in three of the pairs, it was not possible to discriminate between the compounds of one pair, and it was possible to discriminate between the compounds of the other pair at two out of the four laboratories. The embryo culture results generally show a good correspondence with the embryotoxic properties of the compounds tested in vivo, although the embryo culture method appeared to be able to discriminate between only some of the pairs of chemical analogues. Some discrepancies may have arisen among the laboratories, because of methodological differences. These results suggest that the post-implantation rat embryo culture method may be a useful tool for screening xenobiotics within classes of compounds known to interfere with embryogenesis during the period of development represented in culture.

Platzek T, Bochert G. **Embryotoxicity induced by alkylating agents: 10. Analysis of the combined teratogenic effects of methylnitrosourea and ethylmethanesulfonate in mice.** *Teratog Carcinog Mutagen* 1995;15(4):201-14.

In previous studies the direct-acting alkylating model compounds methylnitrosourea (MNU) and ethylmethanesulfonate (EMS) were investigated with regard to dose-response of teratogenicity as well as DNA adduct formation in mice. In this study the teratogenic effects induced by combined treatment with these substances were analyzed using doses which, following single treatment with either substance, were around the threshold level, i.e., no adverse effect level (NOAEL) and lowest observed adverse effect level (LAOEL). Combined treatment of LAOELs resulted in a threshold-like response, while the combination of the NOAEL of one substance with the LAOEL of the other increased the response rate dramatically to nearly 100%. This phenomenon was further evaluated using biometrical methods. The dose-response surface as well as isobolograms were calculated in order to describe the combination effect. In addition, a dose-response model was fitted to the data. In conclusion, the initially surprising high combination effect revealed to be not so extraordinary when considering the steepness of the dose-response relationships of the single substances.

Propst TL, Fort DJ, Stover EL, Schrock B, Bantle JA. **Evaluation of the developmental toxicity of benzo[alpha]pyrene and 2-acetylaminofluorene using Xenopus: modes of biotransformation. Stover Group.** *Drug Chem Toxicol* 1997 Feb-May;20(1-2):45-61.

The developmental toxicities of benzo[alpha]pyrene (BAP) and 2-acetylaminofluorene (AAF) were evaluated using FETAX (Frog Embryo Teratogenesis Assay-Xenopus). Young *X. laevis* embryos were exposed to these two compounds 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 cimetidine (CIM), ellipticine (ELL), or alpha-naphthoflavone (alpha-N) to selectively modulate cytochrome P-450 activity. Bioactivation of both of these compounds was indicated by increased developmental toxicity observed in MAS tests. Results obtained in treated MAS tests indicated that BAP was predominantly activated by Cytochrome P-450 isozyme CYP1A1. AAF bioactivation was shown to be only partly mediated by CYP1A1/2. Detoxification pathways for these two compounds were investigated by treatment of the MAS with cyclohexene oxide (CHO) and diethyl maleate (DM) to inhibit the epoxide hydroxylase and glutathione conjugation pathways, respectively. Results indicated

that epoxide hydroxylase was primarily responsible for the detoxification of BAP, with glutathione conjugation playing a secondary role. Detoxification of AAF by these two pathways was not indicated.

Radatz M, Ehlers K, Yagen B, Bialer M, Nau H. **Valnoctamide, valpromide and valnoctic acid are much less teratogenic in mice than valproic acid.** *Epilepsy Res* 1998;30(1):41-8.

The teratogenic properties of valproic acid (VPA) and its analogues depend to a great extent on their chemical structure. We investigated the structure-teratogenicity relationships of VPA, its structural isomer, valnoctic acid (VCA), and their two amide analogues, valpromide (VPD) and valnoctamide (VCD), respectively. Each substance was injected (3 mmol/kg) in NMRI-mice on the morning of day 8 of gestation. Embryo lethality, fetal weight and exencephaly rates were recorded on day 18 of gestation. VPA caused 53% exencephaly, VPD induced 6%, VCA and VCD produced only 1% exencephaly (control values between 0 and 1%). VPA-treated mice also had increased embryo lethality rates (52%). There was no significant change of embryo lethality in the other treatment groups. Pharmacokinetic studies showed that VCD was eliminated from plasma at a slower rate than VPA. Also, the residual teratogenic activity of VPD was not accounted for by the relatively small amounts of its hydrolysis product VPA. This study indicates that VPD, VCA and VCD were distinctly less teratogenic than VPA. Apparently the amidation of the free carboxylic group and/or methyl-substitution at the beta-position of the carbon chain greatly decreased the teratogenic activity of VPA.

Rahman ME, Ishikawa H, Watanabe Y, Endo A. **Carpal and tarsal bone development is highly sensitive to three antiproliferative teratogens in mice.** *Reprod Toxicol* 1996 Nov-Dec;10(6):485-9. When pregnant mice were given small doses of teratogens (cytosine arabinoside, mitomycin C, or busulfan) that did not induce anomalies of any other organs, a high incidence of carpal and tarsal bone anomalies still occurred. The carpal and tarsal bones may be used as a sensitive target for teratogenicity testing.

Rengel D, Pisano A, Alonso D. **Teratogenic action of hydroxyurea during the development of Bufo arenarum.** *Comun Biol* 1995 Sep;13(3):221-31.

Hydroxyurea is an inhibitor of enzymes involved in DNA synthesis. Amphibians have rarely been used to study the effect of this drug during embryonic development. We have kept chronically, two cell and beginning of dorsal blastopore lip stages of *Bufo arenarum* in 0.3, 0.6 and 0.9 mg/mL doses of hydroxyurea. Besides, we subjected as from the two cell stage, ensuing embryonic stages to 0.1 up to 0.5 mg/mL doses of hydroxyurea, for 96 hours. In amphibians, cell division proved higher during the first 48 hours. Our hypothesis is that this inhibitor causes an anomalous and disordered segmentation in *Bufo arenarum*, plus the teratogenic processes observed during morphogenesis. The results show that this DNA inhibitor could have as specific targets, the segmentation stages that affect embryogenesis.

Richard AM, Hunter ES 3rd. **Quantitative structure-activity relationships for the developmental toxicity of haloacetic acids in mammalian whole embryo culture.** *Teratology* 1996 Jun;53(6):352-60. Developmental toxicity in mouse whole embryo culture assay has been reported for acetic acid (AA) and a series of ten haloacetic acids, including mono-, di-, tri-fluoro (MFA, DFA, TFA), chloro (MCA, DCA, TCA), bromo (MBA, DBA, TBA), and monoiodo (MIA) acetic acids. Benchmark concentrations (BC_m), calculated as the lower 95% confidence limit of molar acid concentration producing a 5%

increase in embryos with neural tube defects, provided potency estimates for development of quantitative structure-activity relationships (QSARs). The best overall regression was obtained for the ten halo-acids (excluding AA) and related $\log(1/BC_m)$ to the energy of the lowest unoccupied molecular orbital (Elumo) and acid dissociation constant (pKa) with a correlation coefficient of $r = 0.97$, and a sample size-adjusted $r^2 = 0.92$. This QSAR suggested a common basis for the mechanism of HA activity, which would imply additivity for mixtures of these acids. Examination of QSARs for subsets of the total data set (e.g., monohaloacids) highlighted parameter relationships embedded in the total QSAR, helping to unravel the separate contributions of Elumo and pKa to the overall potency. The relevance of these parameters is discussed in terms of postulated mechanisms of developmental toxicity involving changes in intercellular pH and redox metabolism. The whole embryo assay results pertain to direct embryo exposure and toxicity without the confounding influence of maternal factors. The resulting QSAR model offers possible insight into the mechanism of embryo toxicity that will hopefully contribute to understanding of the more complex, in vivo teratogenicity problem.

Robert E. [**Example of teratogen detection using a birth defects registry: depakine and spina bifida**]. Rev Epidemiol Sante Publique 1996;44 Suppl 1:S78-81. (Fre)

Most of the human teratogens were discovered as a result of case reports and not primarily through epidemiological studies. We report the detection of a teratogenic effect based both clinical experience and epidemiological analysis. The possibility that anticonvulsant drugs might be teratogenic has been discussed in the literature for several decades. In 1982, we suggested a specific association between valproic acid and spina bifida. The observation was coming both from personal interviews with families of liveborn infants operated upon for spina bifida and from registry data: among 200,000 infants born in the Rhone-Alpes region between August 1979 and August 1982, 11 had spina bifida and were born to epileptic women, 9 of them treated with valproate. Further data confirmed these findings and led to an estimation of about 1-2% for the risk to have an infant with spina bifida after use of valproic acid. The detection of valproic acid teratogenicity raised from an unusual set of birth defects registry, a special interest for spina bifida, a questionnaire with a routine question on epilepsy, a high prescription rate of valproic acid (28% of women in childbearing age) and the registry status of member of the International Clearinghouse for Birth Defects Monitoring Systems. From a public health point of view, this finding has led doctors to consider treatment with valproic acid as a new indication for prenatal diagnosis. 20 new cases were prenatally detected in 10 years in the Central East region of France.

Seetharam B, Christensen EI, Moestrup SK, Hammond TG, Verroust PJ. **Identification of rat yolk sac target protein of teratogenic antibodies, gp280, as intrinsic factor-cobalamin receptor**. J Clin Invest 1997 May 15;99(10):2317-22.

Previous studies in the rat have shown that antibodies to gp280, a protein > 200 kD and closely associated with the early endocytic system can induce fetal malformations. Although gp280 is thought to act as a receptor, its ligand(s) is not known. In the current study, we report that purified gp280 from rat kidney, like the intrinsic factor-Cobalamin receptor (IFCR), binds to the intrinsic factor-cobalamin (IFCbl) complex with an association constant of $0.3 \times 10^9 \text{ M}^{-1}$ and mediates its internalization. Furthermore, antibodies raised to purified gp280 and IFCR inhibited the binding of IF-[^{57}Co]Cbl complex to intestinal, renal, and yolk sac apical membranes and revealed a single identically sized protein on immunoblotting of the renal membranes. Both antibodies precipitated a single radiolabeled

protein > 200 kD from cellular extract from [35S]methionine-labeled yolk sac epithelial cells, and antibody to gp280 inhibited the uptake and internalization of 125IF-Cbl. Immunoelectron microscopy using the two antibodies revealed that in the kidney, both proteins were colocalized. These observations suggest that IF-Cbl complex is a ligand for gp280 and that gp280 and IFCR are identical proteins.

Shibata M, Fujino E, Sato Y, Numazawa S, Yoshida T, Kuroiwa Y. **Inhibition of insulin-induced chondrogenic differentiation of embryonic carcinoma cells by a teratogenic compound YM9429.**

Biochem Biophys Res Commun 1996 Aug 14;225(2):593-9.

A teratogenic compound cis-1-[4-(p-menthane 8-yloxy)phenyl]piperidine (YM9429) induces cleft palate selectively in rat fetuses. The effect of YM9429 on chondrogenic differentiation was investigated using mouse embryonic carcinoma ATDC5 cells that produce chondrocyte specific extracellular matrix upon insulin stimulation. YM9429 at concentrations that showed no growth-inhibitory effect on logarithmic proliferating cells suppressed insulin-mediated increases in Alcian blue staining and expression of type II collagen almost completely. Under the identical conditions, insulin-stimulated cell growth was only partially blocked by the compound. The early response genes such as c-fos and c-jun were induced by insulin even in the presence of YM9429. On the other hand, YM9429 inhibited accumulation of cAMP during the differentiation process. These results indicate that YM9429 selectively inhibits in vitro chondrogenic differentiation of ATDC5 cells.

Sjoblom T, West A, Lahdetie J. **Apoptotic response of spermatogenic cells to the germ cell mutagens etoposide, adriamycin, and diepoxybutane.** Environ Mol Mutagen 1998;31(2):133-48.

In testis, apoptosis is a way to eliminate damaged germ cells during their development. In this study, we evaluated the ability of three germ cell mutagens to induce apoptosis (or programmed cell death) at specific stages of rat seminiferous epithelial cycle. These chemicals include the cancer chemotherapy drugs etoposide and adriamycin and the butadiene metabolite diepoxybutane. According to our results, etoposide is a very potent inducer of apoptosis in male rat germ cells and the cell types most sensitive to it include all types of spermatogonia, zygotene, and early pachytene spermatocytes and meiotically dividing spermatocytes. Also, adriamycin causes an increase in apoptosis at specific stages of seminiferous epithelial cycle and the most sensitive cell types are type A3-4 spermatogonia, preleptotene, zygotene, and early pachytene spermatocytes. Diepoxybutane does not cause any significant increase in the frequency of apoptosis in rat testis. In addition, we studied whether p53 is taking part in the apoptotic response of spermatogenic cells by studying the levels of p53 protein in testis before and after chemical treatment. No accumulation of p53 in testis was seen after treatment with these three chemicals. The expression of two p53-regulated genes, p21WAF1 and mdm2, was also studied but no increase in the levels of mRNA of these genes was observed after treatment. The results indicate that apoptosis should be taken into consideration when the genotoxic effects of chemicals are evaluated in germ cells.

Stevens JT, Tobia A, Lamb JC 4th, Tellone C, O'Neal F. **FIFRA Subdivision F testing Guidelines: are these tests adequate to detect potential hormonal activity for crop protection chemicals? Federal Insecticide, Fungicide, and Rodenticide Act.** J Toxicol Environ Health 1997 Apr 11;50(5):415-31.

Recently, a major topic of discussion has been the impact of synthetic chemicals that possess the capacity to alter hormonal activity, the so-called endocrine modulators, with potentially the capacity to

alter the reproductive capability of humans. Particularly, various synthetic pesticides and industrial chemicals that persist in the environment and/or bioaccumulate have been implicated. Further, it has been alleged that the standard tests for pesticide registration as required by the U.S. Environmental Protection Agency (EPA) and other regulatory agencies may be inadequate to detect endocrine modulating effects. To address these shortcomings, it has been proposed that very specific tests for estrogen receptor binding, or in vitro cell response to chemicals, be used to identify potential endocrine modulators. However, such approaches have certain flaws that limit their application as screens. First, very specific tests, like receptor binding, evaluate only a single chemical event per test. Such tests do not measure toxicity or biological response. Isolated systems are very important for studying mechanisms of action or structure activity relationships, but can only provide a preliminary screen for a single mechanism of toxicity. Isolated systems can not be used to regulate a chemical without additional information. Second, they fail to test many other parts of the neuroendocrine control of the reproductive system. Testing for adverse effects in highly specific in vitro systems failed to replace whole-animal models in carcinogenesis and will also fail in reproductive toxicology because this system is too complicated for such as in vitro approach to be accurately predictive. Advanced tests, such as the EPA multigeneration study, are more effective, and reliable means for evaluation than any specific and narrowly focused screening tests. Experience has shown that a better approach to testing chemicals is to evaluate their effects on the whole animal. When one part of the system is adversely affected, various processes may be indirectly affected and can be detected in the animal model. For example, a modulation of testosterone synthesis could lead to (1) altered accessory sex organ morphology, size, and function; (2) decreased sperm counts; and (3) even decreased fertility. These and many other effects would be noted in toxicity studies that are already required for the registration of crop protection chemicals. The developmental and reproductive toxicity guidelines were recently reviewed in a hearing that included the representatives from the EPA, the public, and the Scientific Advisory Panel. The EPA kept the basic study design the same, but added a few new endpoints to further assess chemical-induced effects on reproductive development and function. The review presented herein concentrates on the required Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) testing for pesticides, and demonstrates how the massive arrays of sensitive endocrine endpoints that are delineated in FIFRA Subdivision F have been successfully used to detect both weak and potent hormonally modulating chemicals. For example, (1) diethyl-stilbestrol (DES), which is a potent synthetic therapeutic estrogen, (2) DDT, which is weakly estrogenic but persistent and bioaccumulating, and (3) dioxins, which have antiestrogenic properties, were all found as being hormonally active in tests similar or identical to FIFRA tests. All food-use pesticides have been evaluated using a comprehensive multigeneration reproduction test. Hence, the FIFRA testing procedures have been demonstrated to identify endocrine modulators of sufficient potency to represent a concern to human health.

Teramoto S, Takahashi K, Kikuta M, Kobayashi H. **Potential teratogenicity of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) in micromass in vitro test.** *J Toxicol Environ Health A* 1998;53(8):607-14.

BIOSIS COPYRIGHT: BIOL ABS. 3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), a by-product of wood pulp manufacture and a contaminant of chlorinated drinking water, was investigated for potential teratogenicity using the micromass in vitro test system. Twelve-day rat embryo midbrain (central nervous system, CNS) and limb bud (LB) cells were exposed to MX at concentrations of 1, 2, 5,

or 10 µg/ml in the culture medium with or without S9 mix. Under the experimental conditions, the amount of MX rapidly declined in the culture medium with a half-life of 56 min. Nevertheless, differentiation of CNS and LB cells was significantly inhibited at concentrations of 2 µg/ml or more, when the cells were exposed to MX in the absence of S9 mix. The estimated IC₅₀ was approximately 3 µg/ml for both CNS and LB cell cultures. On the other hand, exposure of CNS and LB cells to MX along with S9 mix did not reduce the number of differentiated foci at any concentrations tested. These results suggest that MX may be a potential direct-acting *in vitro* teratogen.

Toder V, Savion S, Gorivodsky M, Shepshelovich J, Zaslavsky Z, Fein A, Torchinsky A. **Teratogen-induced apoptosis may be affected by immunopotential.** *J Reprod Immunol* 1996 May;30(2-3):173-85.

Intra-uterine immunization of mice with paternal allogeneic or xenogeneic (rat) splenocytes was found to increase embryo tolerance to cyclophosphamide (CP)-induced teratogenesis. As the CP-induced teratogenic effect was shown to be associated with apoptosis, the present study was designed to investigate whether the protective effect of immunopotential may be realized via an alteration of CP-induced apoptosis. Various doses of CP were injected intraperitoneally into ICR mice on day 12 of pregnancy. Intra-uterine immunization with xenogeneic rat splenocytes was carried out 3 weeks before mating. Implantation sites, resorptions, live and dead fetuses, as well as soft tissue anomalies and external malformations, were recorded to evaluate the CP-induced embryotoxic effect. In parallel, flow cytometric analysis and DNA fragmentation assay were used for evaluation of CP-induced apoptosis in limbs, tail and whole embryos. The treatment of mothers with a high dose of CP induced the death of almost all embryos and striking fetal growth retardation in survivors. This strong embryotoxic effect was accompanied by very prominent DNA degradation in cells collected from whole embryos.

Immunostimulation caused a dramatic decrease of embryonal loss (by approximately 50%) and a significant (about 30%) increase in fetal weight. Such an increase in fetal survival and in fetal weight was found to be accompanied by a clear decrease in apoptosis level in embryo cell population as judged by DNA gel electrophoresis with subsequent quantitation of DNA fragmentation in negatives by an image analysis technique. After treatment with a low dose of CP, a decrease in the proportion of fetuses with limb and tail anomalies in immunized females was accompanied by a decrease in the proportion of apoptotic nuclei in cells taken from limbs and tails. The results of this study suggest that the teratogen-induced apoptosis may, at least partly, be dependent on fetomaternal immune interactions.

Tzimas G, Sass JO, Ruhl R, Foerster M, Klug S, Collins MD, Nau H. **Proximate retinoid teratogens.** *Retinoids* 1994;:17-95.

The metabolism of retinoids is extensive and complex in all species examined. Considerations of toxicokinetics and metabolic aspects can assist in the interpretation of species differences of retinoid teratogenesis and also further our understanding of the mechanism of retinoid action. The large species difference in the teratogenesis of 13-*cis*-retinoic acid can be explained by both kinetic and metabolic aspects: the low sensitivity of the mouse and rat results from low degree of placental transfer of this drug and its 4-oxo metabolite and the high clearance in the maternal organism, predominantly due to beta-glucuronidation; on the other hand, the higher sensitivity of the rabbit may be due to slow elimination of this drug and metabolism to the 4-oxo metabolite, resulting in prolonged exposure of the embryo to these two 13-*cis* isomers. A comparison of the embryonic exposure following approximately equipotent

dosing with 13-cis- and all-trans-retinoic acid (rat, rabbit) indicates that the 13-cis-retinoic acid and its 4-oxo metabolite may be directly-acting teratogens exhibiting intrinsic teratogenic activity: the exposure to all-trans-retinoic acid following administration of the 13-cis isomer is insufficient to account for the teratogenic effects observed. Similar results had previously been obtained for vitamin A (administered as retinyl palmitate or retinol) in the mouse, rat and rabbit: also here, the amount of retinoic acids produced could not explain the teratogenicity of vitamin A, implicating vitamin A (retinol, retinyl esters) as proximate teratogens. In vitro studies with embryonic palate and thymus cultures indicate that all-trans-retinoyl-beta-glucuronide was less potent in inducing differentiation of thymocytes and inhibiting palate fusion, although high concentrations of this glycoconjugate were reached in the cultured tissues; all-trans-retinoic acid may be the proximate metabolite of the beta-glucuronide in regard to these effects. The lower effects exerted by all-trans-retinoyl-beta-glucuronide in vitro, together with the low placental transfer in vivo, suggest that this and other glycoconjugates may be interesting new retinoids with low teratogenic potential.

Tzimas G, Thiel R, Chahoud I, Nau H. **The area under the concentration-time curve of all-trans-retinoic acid is the most suitable pharmacokinetic correlate to the embryotoxicity of this retinoid in the rat.** Toxicol Appl Pharmacol 1997 Apr;143(2):436-44.

Earlier studies with etretinate and its metabolite acitretin suggested that area under the concentration-time curve (AUC) is the most suitable pharmacokinetic correlate to etretinate-induced teratogenesis. In an attempt to test this hypothesis with respect to the embryotoxic effects of all-trans-retinoic acid (all-trans-RA), we determined the embryotoxicity and plasma pharmacokinetics of all-trans-RA and its metabolites following administration of all-trans-RA to Wistar rats on Gestational Day (GD) 9, either subcutaneously (sc; dose levels 1, 3, or 5 mg/kg body mass) or orally (po; 5 mg/kg body mass). The 5 mg/kg dose of all-trans-RA was not embryotoxic when administered orally but led to high rates of embryoletality and skeletal defects following sc treatment. Determination of retinoids by HPLC showed that all-trans-RA reached similar maximum plasma concentrations (C(max)) after both dosing regimens, but its plasma AUC was ca. threefold higher after sc injection than po administration due to the slower uptake rate of the drug and its limited detoxification via beta-glucuronidation following sc injection. Furthermore, retinoid analysis in rat tissues (liver, kidney, duodenum, and jejunum), collected 1 hr after sc or po administration of 5 mg all-trans-RA/kg body mass on GD 9, confirmed that formation of all-trans-retinoyl-beta-glucuronide was much more extensive after po than after sc administration. Finally, linear regression analysis of either C(max). or AUC values of all-trans-RA in rat plasma and fetal abnormality rates showed that AUC values are better correlated with the embryotoxic outcome than C(max) [AUC-based correlation coefficient (r) > 0.90; C(max)-based r < 0.43]. Our findings establish the relevance of the AUC of all-trans-RA, and not its C(max), as the most appropriate pharmacokinetic marker of embryonic exposure and embryotoxic potency of all-trans-RA and stress the importance of the duration of exposure as a major determinant of embryotoxic outcome for retinoids.

Uretsky ME, Rahwan RG. **Problems of conditioning *Xenopus laevis* tadpoles with standard avoidance-response learning paradigms.** Psychol Rep 1996 Dec;79(3 Pt 1):763-73.

The amphibian *Xenopus laevis* embryo (tadpole) provides a satisfactory alternative to mammalian screening for structural teratogens. Testing was undertaken to extend the usefulness of this species for behavioral teratogenicity testing. One simple and eight operant conditioning paradigms were examined:

none elicited learning in *Xenopus* embryos. Adaptation to the conditioning stimulus (light) and freezing in response to the unconditioned stimulus (shock) were responses incompatible with conditioned learning.

Usami M, Ohno Y. **Teratogenic effects of selenium compounds on cultured postimplantation rat embryos.** *Teratog Carcinog Mutagen* 1996;16(1):27-36.

Embryonic susceptibility to selenium (Se) teratogenicity was examined in rats using postimplantation embryo culture. Rat embryos at day 9.5 of gestation were cultured by the roller bottle method for 48 hr in the presence of Se compounds. Sodium selenite, sodium selenate, seleno-DL-methionine, and seleno-DL-cystine were embryo-lethal at 20, 300, 1,000, and 1,000 microM Se, respectively. All of these compounds caused abnormalities such as deformed optic vesicle and swollen rhombencephalon in the viable embryos. These abnormalities were considered to correspond to *in vivo* malformations caused by Se in hamster fetuses or in bird embryos. These results indicate that rat embryos are susceptible to Se teratogenicity. It seems that there are differences in potency ranking of Se compounds between rat and bird embryos.

Vandersea MW, Fleming P, McCarthy RA, Smith DG. **Fin duplications and deletions induced by disruption of retinoic acid signaling.** *Dev Genes Evol* 1998;208(2):61-8.

Retinoic acid (RA), a derivative of vitamin A, plays a critical role as a signaling molecule in axial patterning of vertebrates. Here we report that RA exposure of zebrafish (*Danio rerio*) and mummichog (*Fundulus heteroclitus*) embryos during gastrulation results in homeotic duplications of the pectoral fins in up to 94% of fish. We have observed three to four pairs of fins in an individual fish. Although some duplications are partial, many represent complete axial duplications of the pectoral girdle and fin and include coracoscapulae, proximal radials, and dermal fin elements. Fin duplications are observed only at a defined dose of RA. Inhibition of RA synthesis by exposure to citral during a narrow developmental window leads to fish which lack pectoral fins but can be rescued by addition of exogenous RA, suggesting that RA signaling is critical to fin specification during early development. The ability to consistently induce multiple fins in a large number of vertebrate embryos should contribute to the understanding of genetic regulation of the normal positioning of limbs during embryogenesis.

Watanabe T, Iwase T. **Developmental and dysmorphogenic effects of glufosinate ammonium on mouse embryos in culture.** *Teratog Carcinog Mutagen* 1996;16(6):287-99.

The effects of glufosinate ammonium on embryonic development in mice were examined using whole embryo and micromass cultures of midbrain and limb bud cells. In day 8 embryos cultured for 48 hr, glufosinate caused significant overall embryonic growth retardation and increased embryo-lethality to 37.5% at 10 micrograms/ml (5.0×10^{-5} M). All embryos in the treated groups exhibited specific morphological defects including hypoplasia of the prosencephalon (forebrain) (100%) and visceral arches (100%). In day 10 embryos cultured for 24 hr, glufosinate significantly reduced the crown-rump length and the number of somite pairs, and produced a high incidence of morphological defects (84.6%) at 10 micrograms/ml. These embryos were characterized by blister in the lateral head (100%), hypoplasia of prosencephalon (57.1%), and cleft lips (42.9%) at 20 micrograms/ml (10.0×10^{-5} M). Histological examination of the treated embryos showed numerous cell death (pyknotic debris) present throughout the neuroepithelium in the brain vesicle and neural tube, but did not involve the underlying

mesechyme. In micromass culture, glufosinate inhibited the differentiation of midbrain cells in day 12 embryos with 50% inhibition occurring at 0.55 microgram/ml (2.8×10^{-6} M). The ratios of 50% inhibition concentration for cell proliferation to cell differentiation in limb bud cells were 0.76 and 1.52 in day 11 and 12 embryos, respectively. These findings indicate that glufosinate ammonium is embryotoxic in vitro. In addition to causing growth retardation, glufosinate specifically affected the neuroepithelium of the brain vesicle and neural tube, leading to neuroepithelial cell death.

Welsch F. **Background for two perspectives on the status and prospects of in vitro and short-term prescreening tests for developmental toxicity hazard potential.** *Teratology* 1996 Jun;53(6):332-3.

Zakeri ZF, Ahuja HS. **Cell death/apoptosis: normal, chemically induced, and teratogenic effect.** *Mutat Res* 1997;396(1-2):149-61.

BIOSIS COPYRIGHT: BIOL ABS. Cell death is an integral part of a variety of biological processes including cell proliferation, differentiation, and morphogenesis. We review here the morphological and biochemical nature as well as the genetic basis for cell death during normal and abnormal development. Most often referred to in normal development as programmed cell death, this controlled process determines the size, patterning, and function of many tissues. The importance of its proper genetic regulation is demonstrated by the discovery of cell death-specific genes and the several disorders including cancer and teratogenesis that result from repression or enhancement of cell death. In our studies we employed the developing mouse limb, which provides a defined window of active cell death, to elucidate mechanisms of cell death. We have developed markers that reveal in the developing normal limb an apoptotic morphology with phagocytosis and DNA fragmentation. In the limb deformity mutant Hammertoe there is a defective (restricted) cell death pattern, but the morphology remains apoptotic. By the use of these markers, we were able to observe that the teratogen retinoic acid produced enhanced apoptotic cell death. Most interestingly, retinoic acid-induced cell death in the Hammertoe mutant resulted in correction of the mutant phenotype. Future studies will determine the relationship between exogenous agents and endogenous signaling pathways as well as indicate how these interactions can alter the fate of a given cell and potentially ameliorate a genetic abnormality.

MISCELLANEOUS

Althoff KD, Bergmann R, Wess S, Manago M, Auriol E, Larichev OI, Bolotov A, Zhuravlev YI, Gurov SI. **Case-based reasoning for medical decision support tasks: the Inreca approach.** *Artif Intell Med* 1998;12(1):25-41.

We describe an approach for developing knowledge-based medical decision support systems based on the new technology of case-based reasoning. This work is based on the results of the Inreca European project and preliminary results from the Inreca + project which mainly deals with medical applications. One goal was to start from case-based reasoning technology for technical diagnosis and 'scale-up' to more general non-technical decision support tasks as typically given in medical domains. Inreca technology has been used to build an initial decision support system at the Russian Toxicology Information and Advisory Center in Moscow for diagnosing poison cases caused by psychotropes.

Cassee FR, Groten JP, Van Bladeren PJ, Feron VJ. **Toxicological evaluation and risk assessment of**

chemical mixtures. Crit Rev Toxicol 1998;28(1):73-101.

A major objective of combination toxicology is to establish whether a mixture of chemicals will result in an effect similar to that expected on the basis of additivity. This requires understanding of the basic concepts of the combined toxicological action of the compounds of the mixture: simple similar action (dose addition), simple dissimilar action (effect or response addition), and interaction (synergism, potentiation, antagonism). The number of possible combinations of chemicals is innumerable, and in vivo testing of these mixtures is unattainable from an ethical, economical, or pragmatic perspective. Prediction of the effect of a mixture based on the knowledge of each of the constituents requires detailed information on the composition of the mixture, exposure level, mechanism of action, and receptor of the individual compounds. Often, such information is not or is only partially available and additional studies are needed. Research strategies and methods to assess joint action or interaction of chemicals in mixtures such as whole mixture testing, physiologically based toxicokinetic modeling and isobologram and dose response surface analyses are discussed. Guidance is given for risk assessment of both simple and complex mixtures. We hypothesize that, as a rule, exposure to mixtures of chemicals at (low) non-toxic doses of the individual constituents is of no health concern. To verify the hypothesis is a challenge; to timely detect exceptions to the rule is the real challenge of major practical importance.

Corbett TH, Valeriote FA, Demchik L, Lowichik N, Polin L, Panchapor C, Pugh S, White K, Kushner J, Rake J, et al. **Discovery of cryptophycin-1 and BCN-183577: examples of strategies and problems in the detection of antitumor activity in mice.** Invest New Drugs 1997;15(3):207-18.

Historically, many new anticancer agents were first detected in a prescreen; usually consisting of a molecular/biochemical target or a cellular cytotoxicity assay. The agent then progressed to in vivo evaluation against transplanted human or mouse tumors. If the investigator had a large drug supply and ample resources, multiple tests were possible, with variations in tumor models, tumor and drug routes, dose-decrements, dose-schedules, number of groups, etc. However, in most large programs involving several hundred in vivo tests yearly, resource limitations and drug supply limitations have usually dictated a single trial. Under such restrictive conditions, we have implemented a flexible in vivo testing protocol. With this strategy, the tumor model is dictated by in vitro cellular sensitivity; drug route by water solubility (with water soluble agents injected intravenously); dosage decrement by drug supply, dose-schedule by toxicities encountered, etc. In this flexible design, many treatment parameters can be changed during the course of treatment (e.g., dose and schedule). The discovery of two active agents are presented (Cryptophycin-1, and Thioxanthone BCN 183577). Both were discovered by the intravenous route of administration. Both would have been missed if they were tested intraperitoneally, the usual drug route used in discovery protocols. It is also likely that they would have been missed with an easy to execute fixed protocol design, even if injected i.v.

Rio B, Lautraite S, Parent-Massin D. **In vitro toxicity of trichothecenes on human erythroblastic progenitors.** Hum Exp Toxicol 1997;16(11):673-9.

Trichothecenes are mycotoxins produced by various species of fungi which can occur on various agricultural products. Among these compounds, T-2 toxin, HT-2 toxin, diacetoxyscirpenol (DAS) and deoxynivalenol (DON) are the most naturally encountered and potent trichothecenes. Consumption of trichothecene contaminated foods by farm animals and humans leads to mycotoxicoses. Trichothecenes are known to induce haematologic disorders such as neutropenia, thrombopenia, and aplastic anemia in

human and animals. The aim of our investigations is to explore the effects of trichothecenes on the haematopoietic progenitors. The four trichothecenes previously demonstrated to be strongly cytotoxic for human CFU-GM have been tested on human BFU-E. For this purpose, a culture model of human erythroblastic progenitors (BFU-E) optimized for toxicological studies was used to determine the effects of T-2, HT-2, diacetoxyscirpenol (DAS) and deoxynivalenol (DON) on red blood cell precursor proliferation and differentiation. Results showed that human BFU-E are as sensitive to trichothecenes as human CFU-GM, except for DON, in the range of concentrations tested. Differentiation of erythroblastic progenitors could be perturbed by these mycotoxins. Human erythroblastic progenitors are also a target of trichothecenes.

Safe S. Limitations of the toxic equivalency factor approach for risk assessment of TCDD and related compounds. *Teratog Carcinog Mutagen* 1997-1998;17(4-5):285-304.

BIOSIS COPYRIGHT: BIOL ABS. Halogenated aromatic hydrocarbons (HAHs), such as polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), and dibenzofurans (PCDFs), are industrial compounds or by-products that have been widely identified as environmental contaminants. Hazard and risk assessment of complex HAH mixtures have utilized a toxic equivalency factor (TEF) approach, where the toxic equivalents (TEQs) of any mixture are equal to the sum of the concentration of individual (i) congeners times their potencies (TEFi) relative to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, TEF = 1.0). $TEQ = \sum (PCDD_i) \times (PCDF_i)$ can be readily calculated from analytical data and provides an estimate of the toxicity of any mixture containing HAHs. Several in vivo and in vitro studies with some PCDD/PCDF and PCB mixtures have demonstrated correlations between experimentally determined and calculated TEQs. However, results of several studies have also shown that for specific responses, the TEQ for some HAH mixtures are non-additive. For example, PCB mixtures and individual PCB congeners such as 2,2',4,4',5,5'-hexachlorobiphenyl inhibit toxic and biochemical responses induced by TCDD and related compounds. Another problem associated with hazard and risk assessment of background exposure to HAHs is the relative contribution of trace levels of HAHs (exodioxins) compared to relatively high exposure to naturally occurring aryl hydrocarbon receptor (AhR) agonists, which act through the same mechanistic pathway.

Safe SH. Development validation and problems with the toxic equivalency factor approach for risk assessment of dioxins and related compounds. *J Anim Sci* 1998;76(1):134-41.

BIOSIS COPYRIGHT: BIOL ABS. Polychlorinated dibenzo-p-dioxins (PCDD), dibenzofurans (PCDF), and biphenyls (PCB) are industrial compounds or by-products that have been widely identified as environmental contaminants, and residues have been detected in fish, wildlife, and humans. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD; dioxin) is the most toxic member of this class of halogenated aromatic hydrocarbons (HAH); mechanistic studies indicate that the toxic and biochemical effects associated with exposure to TCDD are mediated via initial binding to the cytosolic aryl hydrocarbon (Ah) receptor protein present in target tissues and organs. Several other 2,3,7, 8-substituted PCDD and PCDF and non-ortho substituted PCB also bind to the Ah receptor and induce toxic responses similar to those for TCDD. Moreover, for these HAH there is a rank order correlation between their structure-Ah receptor binding and structure-toxicity relationships, and this supports a role for the Ah receptor in mediating these responses. Thus, the toxic equivalency factor (TEF) approach for HAH is based on the common mechanism of action for TCDD and related compounds in which a TEF value for a dioxin-like

congener is defined as the potency of the individual (i) congener relative to TCDD ($(EC_{50}(\text{TCDD})/EC_{50}(\text{test compound}))$). The toxic or dioxin equivalent (TEQ) for a mixture of FM is defined by the following equation: $TEQ = \sum (PCDD_i)F_i$ residues contain complex mixtures of HAH (exodioxins) and the TEF/TEQ approach is used to regulate emissions and estimate the potential exposure and possible adverse health effects of exodioxins. The TEF approach for risk assessment of exodioxins makes a number of assumptions, including response additivity for individual compounds in a mixture of HAH. This review documents some of the following problems and limitations of the TEF approach: 1) environmental and food residues of HAH contain non-dioxin-like PCB that exhibit antidioxin activity for some responses; 2) the human diet contains endogenous Ah receptor ligands (endodioxins) such as polynuclear aromatic hydrocarbons (PAH), aromatic amines in cooked foods, indole-3-carbinol (I3C), and related hetero-PAH in cruciferous vegetables. Mass balance and mass potency estimates for human dietary intakes suggest that for some responses the effects of natural or endodioxins may be greater than those of exodioxins; and 3) I3C, a weak Ah receptor agonist, also exhibits Ah receptor antagonist activity, and interactions between I3C and endodioxins may inhibit or inactivate some toxic responses and decrease TEQ_{Exodioxin}.

Williams P, Ryan L. **Design of multiple binary outcome studies with intentionally missing data.** *Biometrics* 1996 Dec;52(4):1498-514.

We discuss the design and analysis of studies involving multiple binary outcomes in which only a subset of these outcomes can be measured on each individual. Such studies with intentionally missing data may arise due to practical or economic constraints; several examples from toxicology serve as illustrations. A global test statistic based on generalized estimating equations is presented and evaluated under a variety of missing patterns and correlation structures. Extensions of the global test statistic to allow for clustered data are also described. The relative efficiency of the global test statistic with missing data relative to that for complete data is investigated, both under a common dose effect alternative and when exposure has differential effects on the multiple endpoints. The implications of these efficiency calculations on study design are explored, and several recommendations are provided.