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

A Bibliography with Abstracts

TO ASSIST IN:

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

PREPARED BY

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

The purpose of these bibliographies on "animal alternatives" is to provide a survey of the literature in a format which facilitates easy scanning. This bibliography includes citations from published articles, books, book chapters, and technical reports. Citations to items in non-English languages are indicated with [ ] around the title. The language is also indicated. Citations with abstracts or annotations relating to the method are organized under subject categories. This publication features citations which deal with methods, tests, assays or procedures which may prove useful in establishing alternatives to the use of intact vertebrates. Citations are selected and compiled through searching various computerized on-line bibliographic databases of the National Library of Medicine, National Institutes of Health.

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Suggestions and comments are welcome.

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Balls M. **Validation of alternative tests in the European Union**. Curr Probl Dermatol 1995;23:265-74.

Barnard ND, Kaufman SR. **Animal research is wasteful and misleading**. Sci Am 1997;276(2):80-2.

Frazier JM. **Interdisciplinary approach to toxicity test development and validation**. Toxicol In Vitro 1995;9 (6):845-9.
BIOSIS COPYRIGHT: BIOL ABS. Toxicological processes are complex interactions of biological systems at various levels of organization. These interactions evolve in time in response to the perturbation resulting from the interaction of the toxicant, or its metabolite, with molecular targets in the organism. The successful development and validation of new in vitro toxicity tests for the toxicological evaluation of chemicals and commercial products require the concerted effort of an interdisciplinary research team. Such a team should consist of the following types of experts: (1) cell physiologist/cell culturist someone who not only can grow cells of various origin but also can investigate the normal state of cells and how in vitro culture conditions affect this state; (2) molecular toxicologist someone who understands the molecular mechanisms of toxicological responses (mechanisms of action) and can experimentally investigate their nature; (3) measurement technologist an expert in instrumental technology and the application of these technologies to the measurement of cellular function and responses; (4) theoretical toxicologist/modeller the integrator for the team who can pull the various aspects of the problem together into a unified picture connecting in vitro and in vivo; (5) chemist/structure-activity expert an expert in chemical structure and its relationship to biological activity to guide the selection of chemicals for investigation; (6) in vivo toxicologist/pathologist the individual who provides contact with reality; (7) kineticist an expert in the kinetics/metabolism of chemicals in biological systems who can experimentally investigate this aspect both in vitro and in vivo; (8) statistician an expert in experimental design and data analysis with the ability to develop new analytical tools to compare in vitro and in vivo data. Most research teams consist of a small group comprising a subset of these areas of expertise and therefore struggle with various aspects of the problem, depending on the pieces missing. It is hoped that the resources of the European Centre for the Validation of Alternative Methods (ECVAM) will be adequate to pull together such an interdisciplinary team to make rapid progress in the development and validation of new testing methodologies.

Hasspieler BM, Haffner GD, Adeli K. **In vitro toxicological methods for environmental health testing**. Rev Environ Health 1996;11(4):213-27.
CBAC COPYRIGHT: CHEM ABS A review with 51 refs. Increasing public interest in environmental health issues has created a demand for alternatives to using animals for assessing the toxic effects of chem. mixts. on humans. This review focuses on applications of in vitro toxicol. screening methods developed for human health biomonitoring using cultured clonal cell lines, which have the which have the following advantages: genetic variation between samples and expts. is minimal; the cultivation of cell lines is rapid and consistent conditions for culture are easily maintained; most of the phenotypic variation that is encountered with use of cell donors is eliminated; and radiolabeled precursors can be used for labeling and quantifying protein and DNA. We describe the current state of development of in vitro toxicity testing methods, present detailed procedures for the test methods optimized in our lab., and compare these techniques with other approaches. Toxicity testing using cell lines provides a mechanism to quantify the risks assocd. with environmental exposure to chem. mixts.

Koeter H. **Validation: a highly charged concept**. Toxicol In Vitro 1995;9(6):851-6.
BIOSIS COPYRIGHT: BIOL ABS. In order that a proposal for an alternative to an animal test be developed as an internationally accepted guideline, there needs to be consensus on the validity of the method proposed. Over the years, considerable attempts have been made to 'validate' promising alternatives. Probably without exception, these validation programs demanded considerable budgets whereas the high expectations as to the output, which would justify the costs involved, were hardly ever met. What went wrong? Obviously, as for each new animal test, each new alternative to an animal test should be subjected to a critical appraisal procedure involving its scientific justification, its sensitivity and its reproducibility, before it could be internationally acceptable. Although there may be differences of opinion on the extent of this exercise, there is considerable agreement that validation in one way or another is essential. None the less, validation programs so far have not resulted in the broad acceptance of any alternative test method. There may be two reasons for this failure. First, the results of the validation studies may have been unsatisfactory, which could mean that either the method subjected to validation failed to show the desired relevance and reliability, or the validation study as such yielded inconclusive results. Secondly, despite clear-cut (supporting) results from the validation exercise, toxicologists/regulators appear reluctant actually to use the data provided for hazard and risk assessment procedures because of a lack of confidence with the (types of) endpoints of the new test. The latter in particular can be considered a major hurdle in the process of acceptance of alternative tests. Therefore, an independent and objective review of any new test, with a view to its usefulness as a contribution to the set of data essential for hazard characterization and risk assessment, should be considered the first step of any comprehensive validation project. Further, the establishment of international centers such as the Johns Hopkins Center for Alternatives to Animal Testing (CAAT) and the European Centre for the Validation of Alternative Methods (ECVAM) where scientists, including regulators, can meet and discuss strategies not only for validation but also for the use of alternative methods in risk assessment, is considered essential for a good understanding of the relevance of the new in vitro, toxicity tests.

Loprieno N, Bruner LH, Carr GJ, Chamberlain M, Cottin M, De Silva O, Kato S. **Alternatives in cosmetics testing**. Toxicol In Vitro 1995;9(6):827-38.
BIOSIS COPYRIGHT: BIOL ABS. This paper represents a summary of presentations made during a round-table discussion at the ECVAM Opening Symposium. After introductory comments on the cosmetic industry's use of alternative methods in the safety assessment process, the use of alternative methods by L'Oreal and by the Japanese cosmetic industry is outlined, current validation studies in Japan are noted, and the involvement of COLIPA, the European Cosmetic, Toiletry and Perfumery Association, in promoting the use of alternative methods is discussed. Two final sections deal with the effect of data variability on the performance of alternative methods in validation studies and on the integrated use of quantitative structure-activity relationship (QSAR) analysis with other approaches in the safety assessment process.

Simmons JE, Gennings C. **Experimental designs, statistics and interpretation**. Food Chem Toxicol 1996;34(11-12): 1169-71.
The Working Group on Experimental Designs, Statistics and Interpretation considered the use of statistics in combination toxicology, the terminology used to describe the interaction(s) of chemicals, the use of efficient experimental designs to minimize animal use, the diverse interests and goals covered by combination toxicology and approaches useful for complex mixtures. The importance of the use of appropriate experimental designs and statistical methodology was recognized. Given the present lack of consensus on terminology and methodology, it is recommended that investigators provide in their publications the definition of additivity and the mathematical model being used.

Svendsen O, Sandoe P, Thorn NA. **Laboratory animal science, welfare and ethics in pharmacology and toxicology**. Pharmacol Toxicol 1997;80(1):3-5.

Umeda M. [**Toxicity tests using cultured cells**]. Jpn J Toxicol Environ Health 1996;42(6):443-52. (Jpn)
BIOSIS COPYRIGHT: BIOL ABS. Toxicological studies using cultured mammalian cells are extremely useful and

expected to be important alternatives to animal tests and experiments. There are various types of cultured cell experiments for various purposes, which need strict step-wise validation studies when used as the routine tests. The following test methods under validation studies are explained here; direct cytotoxicity test, cytotoxicity test using cultured myotubes for intramuscular injection drugs, mouse lymphoma assay for gene mutation, in vitro cell transformation test for carcinogens and tumor promoters, and metabolic cooperation assay for tumor promoters. In order to achieve alternatives of animal experiments, researchers engaging in toxicological studies and recognizing their necessity are required to cooperate together for the further development of the experimental methods.

Yang RS. **Some current approaches for studying combination toxicology in chemical mixtures**. Food Chem Toxicol 1996;34(11-12):1037-44.
Using conventional toxicology methodologies and at the mode and rate of studying chemicals in the last 25 yr, it is doubtful that society will ever have adequate toxicology information on the majority of the chemicals we used now on in the future. Considering further the issue of health effects of chemical mixture exposure (i.e. real-world issues), the problem of not being able to obtain adequate toxicology information is amplified. From a different perspective, concerns over animal rights have raised the consciousness of many biomedical researchers regarding animal experimentation. As many as 17-100 million animals are estimated to be killed for biomedical research in the US alone each year; therefore, minimizing animal usage judiciously in toxicological research should be in the mind of every responsible toxicologist. From these considerations, it is apparent that new, alternative, less animal-intensive, shorter-term and less expensive toxicology methods must be developed if there is to be a reasonable chance to deal with the hundreds of thousand of chemicals, as well as the near-infinite number of chemical mixtures, in the environment. Some of the recent advances indeed are heading towards that direction. In this article, a number of approaches for research work on combination toxicology of chemical mixtures are given; the examples are selected based on one or more of the following criteria: (1) minimizing animal usage; (2) shortening experimental durations; (3) studying environmentally realistic concentrations; (4) utilizing statistical/mathematical modelling; (5) advancing efficient experimental designs and (6) developing predictive toxicology.

CARCINOGENESIS

Alden CL, Smith PF, Piper CE, Brej L. **A critical appraisal of the value of the mouse cancer bioassay in safety assessment**. Toxicol Pathol 1996;24(6):722-5.
Substantial progress in understanding nongenotoxic or secondary mechanisms of tumorigenesis has been made over the past decade However, the methods used in regulated studies for assessment of carcinogenic potential in chemicals in development have not evolved significantly. Based on the experience of over 30 yr of testing and the societal need to control costs, reevaluation of standard cancer rodent bioassay protocols is being done. Expert consensus after evaluating the results of full-scale rodent bioassays of both sexes in 2 species is that a reduced protocol is acceptable. After review of relevant data, it is our opinion that cancer hazard assessment in male and female rats only would be sufficient and that, in the future, mouse bioassays will not add significant value on a routine basis. We are unable to find an example of a mouse tumorigenic finding that predicts a confirmed or probable human response with negative findings in a rat bioassay. The savings realized by eliminating mouse testing from routine protocols would be substantial and better spent in expanding short-term studies to add to our understanding of chemical carcinogenesis.

Badawi AF, Stern SJ, Lang NP, Kadlubar FF. **Cytochrome P-450 and acetyltransferase expression as biomarkers of carcinogen-DNA adduct levels and human cancer susceptibility**. Prog Clin Biol Res 1996;395:109-40.
Carcinogen-DNA adducts are generally regarded as relevant biomarkers of carcinogen exposure and their levels in target tissues have often been predictive of tumor incidence in experimental animals. Thus, human risk assessment procedures have utilized dose-response models that assume proportional relationships between carcinogen

exposure and cancer susceptibility, even though wide inter-individual variations in human metabolic activating enzymes have now been clearly established. To evaluate these approaches, we have examined the relationship between carcinogen exposure, DNA adduct levels, metabolic activation phenotypes, and cancers of the larynx, urinary bladder, and colon. Cigarette smoking is a strong risk factor for cancers of the larynx and urinary bladder. In the larynx, the DNA adducts appear to be derived predominantly from polycyclic aromatic hydrocarbons (PAHs) and are evident only in tissue from smokers. However, adduct levels appear to be determined primarily by expression of cytochrome P450 (CYP) 2C9/10, which varies > 10-fold in different individuals. This CYP catalyzes the metabolic activation of benzo (alpha) pyrene (BP) to a 9-hydroxy-BP-DNA adduct that accounts for up to 25% of the putative PAH adducts formed in vivo. For the urinary bladder, putative aromatic amine (AA)-DNA adducts are predominant and are significantly elevated in current smokers. Rapid CYP1A2 and slow acetyltransferase (NAT2) phenotypes have been previously implicated in the activation (N-oxidation) and detoxification (N-acetylation) of AAs for human bladder carcinogenesis. Data now indicate that NAT1, which is expressed in human urothelium and catalyzes the O-acetylation of N-hydroxy arylamines, is significantly correlated with DNA adduct levels and is bimodally distributed in this tissue. Colo-rectal cancer risk, which has been associated with exposure to heterocyclic amines (HAs) in cooked foods, is strongly elevated in individuals with the combined rapid phenotypes for CYP1A2 and NAT2. These enzymes are uniquely responsible for HA N-oxidation and subsequent O-acetylation, forming DNA adducts that are found in human colon. These studies indicate that cancer risk assessment procedures should be redesigned to include biomarkers of susceptibility, especially those involved in carcinogen bioactivation.

Bertram TA. **Aberrant crypt foci: new insights into the early events of colon carcinogenesis induced by genotoxic and nongenotoxic carcinogens** [editorial]. Toxicol Pathol 1996;24(6):782-3.

Bogen KT, Gold LS. **Trichloroethylene cancer risk: simplified calculation of PBPK-based MCLs for cytotoxic end points**. Regul Toxicol Pharmacol 1997;25(1):26-42.
Cancer risk assessments for trichloroethylene (TCE) based on linear extrapolation from bioassay results are questionable in light of new data on TCE's likely mechanism of action involving induced cytotoxicity, for which a threshold-type dose-response model may be more appropriate. Previous studies have shown that if a genotoxic mechanism for TCE is assumed, algebraic methods can considerably simplify the use of physiologically based pharmacokinetic (PBPK) models to estimate virtually safe environmental concentrations for humans based on rodent cancer-bioassay data. We show here how such methods can be extended to the case in which TCE is assumed to induce cancer via cytotoxicity, to estimate environmentally safe concentrations based on rodent toxicity data. These methods can be substituted for the numerical methods typically used to calculate PBPK-effective doses when these are defined as peak concentrations. We selected liver and kidney as plausible target tissues, based on an analysis of rodent TCE-bioassay data and on a review of related data bearing on mechanism. Tumor patterns in rodent bioassays are shown to be consistent with our estimates of PBPK-based, effective cytotoxic doses to mice and rats used in these studies. When used with a margin of exposure of 1000, our method yielded maximum concentration levels for TCE of 16 ppb (87 micrograms/m3) for TCE in air respired 24 hr/day, 700 ppb (3.8 mg/m3) for TCE in air respired for relatively brief daily periods (e.g., 0.5 hr while showering/bathing), and 210 micrograms/liter for TCE in drinking water assuming a daily 2-liter ingestion. Cytotoxic effective doses were also estimated for occupational respiratory exposures. These estimates indicate that the current OSHA permissible exposure limit for TCE would produce metabolite concentrations that exceed an acute no observed adverse effect level for hepatotoxicity in mice. On this basis, the OSHA TCE limit is not expected to be protective.

Duffus JH. **Epidemiology and the identification of metals as human carcinogens**. Sci Prog 1996;79(Pt 4):311-26.
Classification of substances as probable human carcinogens under the current IARC classification scheme is dependent on epidemiological evidence. The epidemiological data relating to the four metals currently identified as probable human carcinogens, in the metallic form or in the compounds, are reviewed and the weaknesses identified. These weaknesses lie mainly in exposure assessment. The weaknesses may be overcome to some extent by the use of metademographic methods as applied recently to the respiratory cancers that occurred at the Clydach Nickel Refinery in the first 30 years of this century. The general conclusion is that the epidemiological data

relating to metals are unsatisfactory bases for the IARC classifications. There is a need to revise these classifications and to make them more precise by identifying exactly the substances which have caused human cancers.

Farmer PB, Cordero R, Autrup H. **Monitoring human exposure to 2-hydroxyethylating carcinogens**. Environ Health Perspect 1996;104(Suppl 3):449-52.
It is known that human hemoglobin contains low levels of N-terminal N-(2-hydroxyethyl)valine. Possible sources of this modified amino acid are exposure to ethylene oxide or other 2-hydroxy-ethylating agents. Although such processes are likely to occur endogenously, the exogenous contribution to the adduct formation is unclear. In order to explore the latter, we have analyzed N-(2-hydroxyethyl)valine in the globin of 49 pregnant women and evaluated the effect of smoking status, area of residence, and glutathione S-transferase M1 genotype on adduct levels. Transplacental transfer of hydroxyethylating agents was also studied by the analysis of umbilical cord hemoglobin. The adduct levels in smokers were significantly higher than those in nonsmokers. The adduct levels in umbilical cord blood globin were quantitatively related to those in maternal blood (maternal:fetal ratio 2.7 in smokers and 2.8 in nonsmokers). In the nonsmokers, there was no statistically significant difference in the adduct level between the urban and rural areas, but the level in suburbia tended to be lower than that in the rural area. In the combined smoker and nonsmoker groups, there was no effect of the glutathione S-transferase M1 genotype on levels of N-(2-hydroxyethyl)valine.

Flato S, Hemminki K, Thunberg E, Georgellis A. **DNA adduct formation in the human nasal mucosa as a biomarker of exposure to environmental mutagens and carcinogens**. Environ Health Perspect 1996;104(Suppl 3):471-3.

Flesher JW, Horn J, Lehner AF. **7-Sulfooxymethyl-12-methylbenz[a]anthracene is an exceptionally reactive electrophilic mutagen and ultimate carcinogen**. Biochem Biophys Res Commun 1997;231(1):144-8.
The hypothesis was tested that an ultimate carcinogen of 7-hydroxymethyl-12-methylbenz[a]anthracene (HMBA), a major metabolite of 7,12-dimethylbenz[a]anthracene (DMBA), is a benzylic carbonium ion generated from an exceptionally reactive aralkylating metabolite, such as an electrophilic sulfate ester. In conformity with this hypothesis, sarcomas were rapidly induced in rats following repeated subcutaneous injection of HMBA (67%).

Flesher JW, Horn J, Lehner AF. **7-Sulfooxymethylbenz[a]anthracene is an ultimate electrophilic and carcinogenic form of 7-hydroxymethylbenz[a]anthracene**. Biochem Biophys Res Commun 1997;231(3):712-6.
The hypothesis was tested that 7-sulfooxymethylbenz[a]anthracene (7-SBA) is an ultimate electrophilic and carcinogenic form of 7-hydroxymethylbenz[a]anthracene. In conformity with this hypothesis, 7-SBA was more carcinogenic than 7-HBA in inducing sarcomas at the site of repeated subcutaneous injection. These metabolites were individually administered to female Sprague-Dawley rats, beginning at 30 days of age, in 0.2 mumol doses given three times each week for 20 doses. One year after the first injection of 7-SBA, seven of thirteen female Sprague-Dawley rats had developed sarcomas. 7-HBA, on the other hand, had induced sarcomas at the site of injection in only two of tweleve rats. No tumors developed either in the control group given sesame oil:DMSO only or in the untreated control group. It would appear from the results summarized here that the search for an ultimate electrophilic and carcinogenic form of 7-HBA has been successful.

Hammons GJ, Milton D, Stepps K, Guengerich FP, Tukey RH, Kadlubar FF. **Metabolism of carcinogenic heterocyclic and aromatic amines by recombinant human cytochrome P450 enzymes**. Carcinogenesis 1997;18(4):851-4.
The N-hydroxylation of carcinogenic arylamines represents an initial step in their metabolic activation. Animal studies have shown that this reaction is catalyzed by the cytochrome P450 (P450) enzymes P450 1A1 and P450 1A2. In this study, utilizing enzymes expressed in Escherichia coli (and purified) or in human B-lymphoblastoid cells, the catalytic activities of recombinant human P450 1A1, P450 1A2, and P450 3A4 for N-hydroxylation of several carcinogenic arylamines were determined. P450 1A2 from both expression systems catalyzed the N-hydroxylation

of 4-aminobiphenyl and the heterocyclic amines, 2-amino-3-methylimidazo[4,5 -f/quinoline (IQ), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). Rates were similar, with values of 1.1-7.8 nmol/min/nmol P450. In contrast, P450 1A1 catalyzed N-hydroxylation of only PhIP, and no activity was observed with P450 3A4. Further kinetic analysis with purified P450 1A2 showed similar Km and Vmax values for N-hydroxylation of the arylamines. Furafylline and fluvoxamine, inhibitors of P450 1A2 activity in human liver microsomes, were found to be inhibitory of the recombinant P450 1A2 N-hydroxylation activity. Results from this study are supportive of a major role for human P450 1A2 in the metabolic activation of arylamines.

Hayward JJ, Shane BS, Tindall KR, Cunningham ML. **Differential in vivo mutagenicity of the carcinogen/non-carcinogen pair 2,4- and 2,6-diaminotoluene**. Carcinogenesis 1995;16(10):2429-33.
The mutagenic activity of 2,4-diaminotoluene (95807) (2,4DAT) and 2,6-diaminotoluene (823405) (2,6DAT) was examined in a new mouse bioassay. The purpose of the study was to determine if an assay based on Big-Blue-mice, a transgenic strain of B6C3F1-mice that contained multiple copies of bacteriophage-lambda, each with an lacI mutational target gene integrated into its chromosome-4, known as the Big-Blue assay, could be used to detect a difference in mutagenicity between 2,4DAT, a potent rodent hepatocarcinogen, and 2,6DAT which is not carcinogenic. Both compounds had shown equal mutagenic potency in the Ames/Salmonella assay. Big-Blue-mice were administered 0 or 1,000 parts per million 2,4DAT or 2,6DAT in their diet for 30 or 90 days. Other mice were injected intraperitoneally daily for 5 days with 6mg/kg dimethylnitrosamine (DMN) as a positive control. Experimental mice were killed on day 31 or day 91 and control mice were killed 15 days after the last dose. The livers were removed and sectioned. The genomic DNA was extracted from the sections and the frequency of mutations at the lacI locus was determined. No significant differences in lacI mutation frequency were seen between 2,4DAT and 2,6DAT treated mice and the negative controls on day 31. After 90 days of exposure, the frequency of lacI mutations in 2,4DAT treated mice was significantly greater than in 2,6DAT treated mice and the negative controls, 12.1x10(-5) versus 5.6x10(-5) and 5.7x10(-5), respectively. The mutation frequency in DMN treated mice averaged 3.12 times that of the other groups at both time points. The authors conclude that the Big-Blue assay is capable of discriminating between two compounds which have differing carcinogenic properties, but which have shown similar mutagenicity in the Ames/Salmonella assay; is sensitive to mutagens through subchronic dietary exposure; and yields a differential response which is dependent upon the length of time mice are exposed to a mutagen.

Holt PD. **Consideration of tissue response in the application of the two-mutation model to radiation carcinogenesis**. Int J Radiat Biol 1997;71(2):203-13.
The Moolgavkar-Venzon-Knudson (MVK) two-mutation model of carcinogenesis is an analytical model that predicts the variation of cancer yield-rate with time, and with dose of a carcinogen The model is biologically based, and assumes that a specific mutation in a stem-cell will increase its rate of proliferation compared with that of unmutated cells, so that a clone of pre-malignant cells develops; a second specific mutation in any one of these will make it malignant, and a cancer will start to grow. The model has been used in recent years to analyse a number of sets of epidemiological data on carcinogenesis. The purpose of this paper is to point to a problem in the use of this model for radiation-induced carcinogenesis, namely that ionizing radiation causes reproductive death of stem cells, which leads to regenerative division and hence a change in the number of stem-cells at risk. The possible effects of such changes on the predictions of the model are discussed. At low dose-rates of continuous or chronic irradiation and at low doses of acute irradiation, it is expected that pre-malignant cells will be killed along with the unmutated cells, and that the regenerative division of the surviving pre-malignant cells will restore the numbers of both stem cells and pre-malignant cells to what they would have been in the absence of cell killing; hence, no net effect of the tissue regeneration is expected. At high dose-rates, the initial delay in regenerative division and subsequent faster proliferation are expected to lead to an initial reduction in tumour yield-rate with time (compared with that predicted by the MVK model) followed by a faster increase. For acute irradiation, in the particular case of beta-particle irradiation of the skin, at high doses where there are practically no surviving cells in the irradiated area, repopulation by unirradiated cells from the margin is predicted to lead to a decrease in tumour yield-rate with dose. The predictions have been compared with published data on the induction of osteosarcoma in mouse by repeated

injection of 89Sr, the induction of skin tumours in rat by acute and chronic irradiation with electrons, and the induction of skin tumours in mouse by acute irradiation with beta-particles. At low doses and dose-rates the basic MVK model fitted the data well. At higher doses and dose-rates the expected effects of tissue regeneration were observed qualitatively, although there were some discrepancies in detail; these are discussed.

Imaida K, Fukushima S. **Initiation-promotion model for assessment of carcinogenicity: medium-term liver bioassay in rats for rapid detection of carcinogenic agents**. J Toxicol Sci 1996;21(5):483-7.
To bridge the gap between long-term carcinogenicity tests and short-term screening assays, a medium-term liver bioassay system for rapid detection of carcinogenic agents using male F344 rats has been developed. The system is fundamentally based on the two-stage hypothesis of carcinogenesis: initiation with diethylnitrosamine (200 mg/kg, ip) is followed by test chemical administration during the second stage in combination with 2/3 partial hepatectomy. It requires only 8 weeks for the animal treatment and a further few weeks for quantitative analysis of immunohistochemically-demonstrated glutathione S-transferase placental form positive hepatic foci. A total of 277 chemicals have already been analyzed in this laboratory and the efficacy of the system for hepatocarcinogens has thereby been well established. This bioassay is particularly useful for dose-response and chemical mixture studies usually requiring large-scale experiments and also for evaluation of chemopreventive agents. Furthermore, medium-term multi-organ bioassay system, using 5 different chemical carcinogens (DEN, MNU, BBN, DMH and DHPN) has also been established for rapid detection of not only hepatocarcinogens, but also other carcinogens.

Isfort RJ, Leboeuf RA. **The Syrian hamster embryo (SHE) cell transformation system: a biologically relevant in vitro model--with carcinogen predicting capabilities--of in vivo multistage neoplastic transformation**. Crit Rev Oncog 1995;6(3-6):251-60.
Neoplastic transformation is a multistep process that can be modeled in vitro using Syrian hamster embryo (SHE) cells. SHE cells multistage transformation involves several intermediate stages, including morphological transformation, immortality, acquisition of tumorigenicity, and malignant progression. Analysis of the molecular alterations that occur at each stage indicated that morphological transformation results from both carcinogen-induced irreversible chromosomal/genetic mutations and reversible genetic events, including altered DNA methylation. Morphological transformation results from a block in the cellular differentiation of progenitor and determined stem-like cells in the SHE cell population via alternation in the expression of the H19 tumor suppressor gene and other genes. Immortality results from genetic mutations in growth factor responsiveness, including loss of growth suppression by TGF beta and autocrine growth factor production, and genomic stability, resulting in genomic instability and an increased mutation rate. Acquisition of tumorigenicity involves loss of tumor suppressor gene function, altered mitogenic signal transduction, mutation of oncogenes, acquisition of anchorage independent growth, and chromosomal aberrations. Malignant progression is associated with alterations in extracellular matrix growth characteristics, alterations in cytoskeleton structure, elevated fibrinolytic activity, secretion of proteases, and changes in extracellular matrix protein secretion. Together, these changes model the alterations observed during in vivo neoplastic transformation and possibly explain why the SHE assay, as a carcinogen screening tool, is able to identify carcinogens with a 80 to 85% accuracy.

Jiang YH, Lupton JR, Chapkin RS. **Dietary fish oil blocks carcinogen-induced down-regulation of colonic protein kinase C isozymes**. Carcinogenesis 1997;18(2):351-7.
In order to elucidate the influence of dietary constituents on colonic intracellular signal transduction, the effect of different fats on rat colonic epithelial protein kinase C (PKC) alpha (classical), delta (novel) and lambda-zeta (atypical) expression was determined in carcinogen-treated animals. Sprague-Dawley rats were provided with one of two fats (corn oil and fish oil); plus or minus the carcinogen azoxymethane (AOM) and killed at two time points (15 and 37 weeks) in a 2x2x2 factorial design. At 5 and 6 weeks of age, animals were injected s.c. with either AOM at a dose of 15 mg/kg body weight or saline once a week for 2 weeks and continued on the same diet until termination of the study. At 15 and 37 weeks after the second injection, 10 rats from each treatment group were killed. Colonic PKC alpha, delta and lambda-zeta steady-state protein and mRNA levels were determined using immunoblotting and relative quantitative polymerase chain reaction, respectively. Colonic mucosa from rats injected with AOM had significantly suppressed membrane and cytosolic PKC alpha and cytosolic lambda-zeta protein

levels (P < 0.05) as compared to saline-injected control animals at both time points. In contrast, rats fed fish oil diets had significantly higher (P<0.05) Ctosolic PKC delta and lambda-zeta protein levels relative to animals fed corn oil diets. However, the effect of diet and AOM on the steady-state expression of PKC alpha, delta and zeta MRNA was not consistent with changes in the respective isozyme protein levels, suggesting regulation at the post-transcriptional level. These data demonstrate that dietary fish oil blocks the carcinogen-induced decrease in the steady-state levels of colonic musosal PKC Delta and Lambda-zeta, which may in part explain why this fat source protects against colon cancer development.

Kensler TW, Groopman JD. **Carcinogen-DNA and protein adducts: biomarkers for cohort selection and modifiable endpoints in chemoprevention trials**. J Cell Biochem Suppl 1996;25:85-91.
Chemical-specific markers have been developed for a number of environmental carcinogens for use as molecular dosimeters of individual exposure. In addition to contributing substantially to the specificity and sensitivity of epidemiological studies aimed at determining the role of environmental agents in the etiology of human cancers, some of these biomarkers may prove to be useful endpoints for assessing the efficacy of preventive interventions including exposure avoidance or remediation and chemoprevention. Biomarkers of the biologically effective dose may be particularly useful in this context in that they provide a mechanistic linkage between exposure and disease outcome. The biologically effective dose reflects the amount of toxicant that has interacted with its critical molecular target and can be measured through a variety of analytical techniques as either carcinogen-DNA or -protein adducts. Approaches for the development and validation of aflatoxin adduct biomarkers are presented as a paradigm for the application of carcinogen-specific markers for cohort selection and as modifiable endpoints for assessing efficacy in chemoprevention trials.

Klein CB, Costa M. **DNA methylation, heterochromatin and epigenetic carcinogens**. Mutat Res 1997;386 (2):163-80.
This paper will explore emerging concepts related to alternative carcinogenic mechanisms of 'non-mutagenic,' and hence epigenetic, carcinogens that may heritably alter DNA methylation without changing the underlying DNA sequence. In this review, we will touch on the basic concepts of DNA methylation, and will elaborate in greater detail on related topics including chromatin condensation, and heterochromatin spreading that is well known to induce gene silencing by position effect variegation in Drosophila and other species. Data from our model transgenic G12 cell system will be presented to support our hypothesis that certain carcinogens, such as nickel, may be carcinogenic not primarily because of their overt mutability, but rather as the result of their ability to promote DNA hypermethylation of important cancer-related genes. We will conclude with a discussion of the broader relevance of our findings and its application to other so-called 'epigenetic' carcinogens.

Klopman G, Rosenkranz HS. **Toxicity estimation by chemical substructure analysis: the TOX II program**. Toxicol Lett 1995;79(1-3):145-55.
The artificial intelligence program MULTICASE and TOX-II, a program that identifies molecular substructures, can predict the toxicity of new molecules. The MULTICASE program attempts to identify functionality, or toxicophore, associated with a carcinogenic response, then analyzes the influence the rest of the molecule may have on the outcome. The dictionaries created by MULTICASE are further analyzed by the TOX-II program, which identifies toxicophores as linear chains of two to ten nonhydrogen atoms, including a side chain. Then the program separates them into congeneric subsets and determines modulators that could account for lack of activity. Even the most complex sets of chemicals are automatically arranged into accessible subsets with common functionality and presumed mechanisms of action. In 4 years, 70 specialized dictionaries have been created by the authors. The capabilities of TOX-II were further extended by adding a metabolism program, META. META seeks metabolizable substructures and applies predetermined transformation rules to the substructure, such as a substrate metabolized by an enzyme and the resulting product. The authors conclude that predictions from TOX-II should be taken as a guide to predict toxicity, not as an absolute determination. Limitations include interspecies differences that could result in prediction uncertainties.

Kubota T, Kase S, Otani Y, Watanabe M, Teramoto T, Kitajima M. **Interferons alpha-2a and beta increase the antitumor activity, detected by MTT assay, of 5-fluorouracil against experimental and clinical human gastrointestinal carcinomas**. Anticancer Res 1997;17(1b):725-8.
In order to investigate the combined antitumor activity of 5-fluorouracil (5-FU), and recombinant human interferon alpha 2a (IFN alpha) or human fibroblastoid interferon beta (IFN beta), the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide (MTT) assay was carried out using a cultured human colon cancer cell line (C-1) and fresh surgical specimens of gastric and colon carcinomas. IFNs did not show positive antitumor activity against C-1 cells, whereas 5-FU showed time- and concentration-dependent antitumor activity against C-1 cells. Furthermore, the antitumor activity of 5-FU on C-1 cells was augmented by IFN alpha or beta. When 5-FU (50 micrograms/ml) with IFN alpha (50 IU/ml). or IFN beta (50 IU/ml) was applied for the MTT assay with 48 hours incubation of fresh surgical specimens of gastric and colon carcinomas, the inhibition rates increased by 10% in 9 of 21 gastric specimens and in 18 of 36 colon carcinomas for IFN alpha (47.4% or 27/57), and in 8 of 15 gastric specimens and in 15 of 28 colon carcinomas for IFN beta (53.5% or 23/43). These results suggest that the chemosensitivity to 5-FU of human gastric and colon carcinomas is increased in the presence of IFNs, without involvement of the host-mediated immune system, and that this combined effect can be predicted by the MTT assay in vitro.

Lefevre PA, Tinwell H, Ashby J. **Mutagenicity of the potent rat hepatocarcinogen 6BT to the liver of transgenic (lacI) rats: consideration of a reduced mutation assay protocol**. Mutagenesis 1997;12(1):45-7. 6-(p-dimethylaminophenylazo)benzothiazole (6BT) is an unusually potent rat hepatocarcinogen, producing large malignant liver tumours after only 2-3 months of dietary administration in a riboflavin-deficient diet. This azocarcinogen has been evaluated in a Big Blue F344 transgenic rat (lacI) gene mutation assay. In a reproduction of the early stages of the carcinogenesis bioassay.

Lewis DF, Ioannides C, Parke DV. **COMPACT and molecular structure in toxicity assessment: a prospective evaluation of 30 chemicals currently being tested for rodent carcinogenicity by the NCI/NTP**. Environ Health Perspect 1996;104(5):1011-6.
A new series of 30 miscellaneous National Toxicology Program chemicals has been evaluated prospectively for carcinogenicity and overt toxicity by COMPACT (Computer Optimised Molecular Parametric Analysis for Chemical Toxicity. CYP1A and CYP2E1). Evaluations were also made by Hazardexpert, and for metal ion redox potentials; and these, together with COMPACT, were compared with results from the Ames test for mutagenicity in Salmonella, the micronucleus test, and 90-day subchronic rodent pathology. Seven of the 30 chemicals (nitromethane, chloroprene, xylenesulphonic acid, furfuryl alcohol, anthraquinone, emodin, cinnamaldehyde) were positive for potential carcinogenicity in the COMPACT evaluation; xylenesulphonic acid and furfuryl alcohol were only equivocally positive. Four of the 30 chemicals-scopolamine, D&C Yellow No. 11, citral cinnamaldehyde-were positive by Hazardexpert; 6 of 30-D&C Yellow No. 11, 1-chloro-2-propanol, anthraquinone, emodin, sodium nitrite, cinnamaldehyde-were positive in the Ames test; 2 of 30-phenolphthalein and emodin-were positive in the in vivo cytogenetics test; and 3 of 30-molybdenum trioxide, gallium arsenide, vanadium pentoxide-were metal compounds with redox potentials of the metal/metal ion indicative of possible carcinogenicity. The overall prediction for carcinogenicity was positive for 12 of 30 chemicals: nitromethane, chloroprene, D&C Yellow No. 11, molybdenum trioxide, 1-chloro-2-propanol, furfuryl alcohol, gallium arsenide, anthraquinone, emodin, sodium nitrite, cinnamaldehyde, vanadium pentoxide). This overall prediction has been made on the basis of the results of the computer tests and from consideration of the information from bacterial mutagenicity, together with likely lipid solubility and pathways of metabolism and elimination.

Lohman FP, Medema JK, Gibbs S, Ponec M, Van De Putte P, Backendorf C. **Expression of the SPRR cornification genes is differentially affected by carcinogenic transformation**. Exp Cell Res 1997;231(1):141-8.
The small proline rich protein (SPRR) genes constitute a family of conserved genes which are part of the human epidermal differentiation complex (EDC) on chromosome 1q21 and code for precursor proteins of the cornified cell envelope. The expression of these genes is strictly linked to keratinocyte terminal differentiation both in vivo and in vitro. Here we show that cultured cell lines derived from squamous cell carcinoma (SCC) show significantly lower levels of SPRR expression than normal human keratinocytes. However, the residual SPRR expression in SCC lines

appears to be both gene and cell line specific. Expression of SPRR2 appears to correlate well with the residual ability of these cells to differentiate. However, the kinetics of SPRR2 expression, following treatment with calcium, an inducer of keratinocyte differentiation, are typical for each cell line and differ substantially from the ones found in normal cells. In most cell lines a rapid transient expression of SPRR2 contrasts with a slow induction leading to a high sustained level of expression in normal cells. This pattern of expression is typical for SPRR2 and not observed for the other SPRR genes or involucrin. Our analysis indicates that the expression of various keratinocyte terminal differentiation markers, even when involved in the same biological process (cornification), can be differentially affected by carcinogenic transformation.

Maciorowski KG, Turner ND, Lupton JR, Chapkin RS, Shermer CL, Ha SD, Ricke SC. **Diet and carcinogen alter the fecal microbial populations of rats**. J Nutr 1997;127(3):449-57.
BIOSIS COPYRIGHT: BIOL ABS. An analysis of viable bacterial populations enumerated on carbohydrate selective media was used to simulate the colonic environment in vitro and determine if differential media could detect significant microbial shifts due to dietary fiber source, dietary fat source, and carcinogen. Male Sprague-Dawley rats were provided with either pectin or cellulose as a fiber source, either corn or fish oil as a source of fatty acids, and injected with either azoxymethane (AOM), a gastrointestinal carcinogen, or saline in a 2 design. At 6 and 10 mo of age, fresh feces were collected, homogenized in anaerobic buffer and anaerobically plated onto differential media. Diets containing pectin supported more anaerobes at 6 mo of age ($P < 0.01$) than diets containing cellulose. Rats injected with AOM and consuming either pectin or corn oil supported more anaerobes at 10 mo of age ($P < 0.05$) than rats injected wtih saline and consuming the same diets. Rats consuming cellulose and receiving AOM but not expressing tumors possessed larger anaerobic populations at 10 mo of age ($P < 0.05$) than rats consuming cellulose, injected with AOM and expressing tumors. These effects show that gastrointestinal bacterial populations as measured by carbohydrate specific media, respond to dietary changes such as dietary fiber source, and thus may play a key role in the etiology of colon cancer.

Makinen M, Forbes PD, Stenback F. **Quinolone antibacterials: a new class of photochemical carcinogens**. J Photochem Photobiol B 1997;37(3):182-7.
Hairless mice were exposed orally to antibiotics of the fluoroquinolone group alone and in combination with irradiation with UVA over an extended period of time to determine the possible skin carcinogenicity in comparison with that with 8-methoxypsoralen, i.e. a known photochemical skin carcinogen. Animals exposed to UVA and fleroxacin, ciprofloxacin, nalidixic acid and ofloxacin exhibited an increase in the number of benign skin tumors when compared with animals exposed to UVA alone. Animals exposed to lomefloxacin and UVA exhibited a specific type of neoplastic progression. In addition to benign papillomas and solar keratoses, a number of cystic squamous cell carcinomas were observed. In the positive control group, which was given 8-methoxypsoralen and UVA, a number of papillomas and superficial squamous cell carcinomas were found. In animals exposed to UVA alone, only a few benign tumors were seen; in unexposed animals, no cutaneous neoplasms were observed. It is concluded that fluoroquinolones warrant further study, because they have potential photocarcinogenic properties.

Marchant CA. **Prediction of rodent carcinogenicity using the DEREK system for 30 chemicals currently being tested by the National Toxicology Program**. The DEREK Collaborative Group. Environ Health Perspect 1996;104(Suppl 5):1065-73.

Maronpot RR, Boorman GA. **The contribution of the mouse in hazard identification studies**. Toxicol Pathol 1996;24(6):726-31.
Because three is usually more extensive toxicity, metabolism, and pharmacokinetic information for pharmaceuticals as opposed to environmental agents, including pesticides, the argument has been made that carcinogenicity testing in two rodent species may not have been necessary for carcinogenicity testing of pharmaceuticals. On the basis of numerical data only, it may be argued that carcinogenicity testing of pharmaceuticals in one species, typically the rat, is sufficient to identify potential human carcinogens. The argument that testing in a second species, typically the mouse, is redundant overlooks the value added by the second species carcinogenicity study. Bioassay data from the second species allows balance and perspective in evaluating the observed effects, and this is especially critical

when there is a marginal, questionable, or inconclusive response in one species. Utilization of two species for carcinogen identification is the principal means for identifying trans-species carcinogens-those mostly likely to be carcinogenic in humans. Given that neither rat nor mouse are ideal surrogates for humans, concordant data from both species strengthens the ability to extrapolate findings to humans. We believe that testing in two species should continue to be the default approach used for carcinogen hazard identification whenever scientifically indicated until such time that acceptable and suitable alternatives are available. To utilize only one species for this important means of protecting human health is premature at this time.

McCann J, Kavet R, Rafferty CN. **Testing electromagnetic fields for potential carcinogenic activity: a critical review of animal models**. Environ Health Perspect 1997;105(Suppl 1):81-103.
BIOSIS COPYRIGHT: BIOL ABS. In order to assess the potential of electromagnetic fields (EMF) to influence the process of carcinogenesis, it will be necessary to supplement epidemiological studies with controlled laboratory studies in animals. There are now a number of suitable assays available that focus on different histopathological forms of cancer and on different stages of carcinogenesis-induction, promotion, progression. In this review we discuss eight major systems in the context of this generalized carcinogenesis paradigm. Our aim is to bring together what is currently known about the biology of carcinogenesis in these systems in order to provide a context for evaluating EMF results as they become available. We also critically discuss EMF test results that have so far been obtained in the animal models reviewed. Most of the 19 completed studies identified were negative. However, suggestive positive results were reported in three promotion assays (in rat mammary gland, in rat liver, and in mouse skin), and in one multigeneration study in mice. Results in the rat liver assay and in the multigeneration study have only been reported in abstract form and cannot be adequately evaluated. Positive results reported in both the rat mammary gland and the mouse skin systems are of weak statistical significance and have not been independently replicated. However, it may be of interest that effects in both systems appear primarily to involve the progression stage of carcinogenesis. We suggest that more definitive conclusions as to the carcinogenic potential of EMF may require expanded test protocols that reinforce traditional carcinogenesis end points with biochemical or other parameters reflective of biological processes known to be associated with carcinogenesis in the different systems.

Moriguchi I, Hirano H, Hirono S. **Prediction of the rodent carcinogenicity of organic compounds from their chemical structures using the FALS method**. Environ Health Perspect 1996;104(Suppl 5):1051-8.
Fuzzy adaptive least-squares (FALS), a pattern recognition method recently developed in our laboratory for correlating structure with activity rating, was used to generate quantitative structure-activity relationship (QSAR) models on the carcinogenicity of organic compounds of several chemical classes. Using the predictive models obtained from the chemical class-based FALS QSAR approach, the rodent carcinogenicity or noncarcinogenicity of a group of organic chemicals currently being tested by the U.S. National Toxicology Program was estimated from their chemical structures.

Nishiyama Y, Tanaka T, Naitoh H, Mori C, Fukumoto M, Hiai H, Toyokuni S. **Overexpression of integrin-associated protein (CD47) in rat kidney treated with a renal carcinogen, ferric nitrilotriacetate**. Jpn J Cancer Res 1997;88(2):120-8.
An iron chelate, ferric nitrilotriacetate (Fe-NTA), induces renal proximal tubular necrosis, a consequence of free radical-associated damage, that ultimately leads to a polycystic change of the renal cortex and a high incidence of renal cell carcinoma (RCC) in rodents. The differential display technique was used to search for inducible genes in the kidney of male Wistar rats treated with Fe-NTA and in the induced RCCs. Six fragments were selected that showed specific quantitative changes in mRNA. Two of them exhibited similar patterns in northern blots as well. One fragment showed a high homology (89%) to murine integrin-associated protein (IAP; CD47). We thus cloned rat IAP cDNA including the entire coding region for use in further analysis. Rat IAP cDNA showed a 21-amino-acid deletion that was also observed in human, but not in mouse. Northern blots revealed that IAP was consistently overexpressed in non-tumorous parts of the kidney (2.4-fold increase, n = 9, P < 0.0001) as compared with matched controls 1 to 2 years after Fe-NTA treatment. IAP overexpression of more than 2.9-fold was found in 25% (2/8) of RCCS studied, and was limited to cases of a high histological grade and lung metastasis. Unexpectedly,

IAP expression was higher in the non-tumorous part of the kidney after Fe-NTA treatment (2.8 fold) than in RCC (1.5-fold) in each case (N = 4, P < 0.05). Abundant expression of IAP MRNA in the renal tubular epithium after Fe-NTA treatment and RCC cells was observerd by in situ hybridization. The results suggest that IAP overexpression my be associated twith Fe-NTA-induced renal cortical tubular damage and regeneration that lead to a polycystic state, and with tumor progression and metastasis induced RCCS.

Oda H, Zhang S, Tsurutani N, Shimizu S, Nakatsuru Y, Aizawa S, Ishikawa T. **Loss of p53 is an early event in induction of brain tumors in mice by transplacental carcinogen exposure**. Cancer Res 1997;57(4):646-50.
BIOSIS COPYRIGHT: BIOL ABS. Experimental carcinogenesis studies using p53-deficient mice have suggested that loss of function of this tumor suppressor gene is generally not an early event but is rather related to tumor progression. However, the biological functions of p53 and the accumulating evidence of alteration in human tumors imply a possible role for loss of p53 in the initial stages of tumorigenesis. Ethylnitrosourea administration to p53-heterozygous pregnant mice resulted in rapid development of primary brain tumors, which are extremely rare in mice, in 70% of the p53-null offspring. Brain tumors also developed later in 4% of heterozygous mice, but they had lost the wild-type allele. Thus, loss of normal p53 gene expression is of direct significance to early events in brain tumorigenesis, and this tumor suppressor gene may protect embryos from DNA damage in the brain induced by transplacental carcinogen exposure.

Okai Y, Higashi-Okai K, Yano Y, Otani S. **All-trans beta-carotene enhances mitogenic responses and ornithine decarboxylase activity of BALB/c 3T3 fibroblast cells induced by tumor promoter and fetal bovine serum but suppresses mutagen-dependent umu C gene expression in Salmonella typhimurium (TA 1535/pSK 1002)**. Cancer Lett 1996;99(1):15-21.
BIOSIS COPYRIGHT: BIOL ABS. Although previous epidemiological studies have indicated that beta-carotene is an important agent for the chemical prevention against carcinogenesis, a recent prospective study has strikingly suggested that supplementation with beta-carotene significantly increased the incidence of some types of cancer (The alpha-Tocopherol and beta-Carotene Cancer Prevention Study Group, New Engl. J. Med., 330 (1994) 1031-1035). To analyze the discrepancy of this problem, the authors analyze the effects of caro biochemical and biological events associated with carcinogenesis by in vitro experiments. (1) All-trans carotene enhanced the proliferation and DNA synthesis of BALB/c 3T3 cells induced by a tumor promoter, 12-O-tetradecanoyl-phorbol-13-acetate (TPA) and fetal bovine serum, although beta-carotene itself did not show mitogenic activity. (2) All-trans beta-carotene caused a remarkable stimulation for the early induction of ornithine decarboxylase (ODC) activity after the stimulation of TPA and fet bovine serum. (3) All-trans beta-carotene exhibited significant antimutagenic activity which suppresses umu C gene expression in Salmonella typhimurium (TA 1535/pSK 1002) induced by a typical mutagen, 2-aminoanthracene (2-AA). These experimental results suggest that all-trans beta-carotene might cause beneficial and harmful effects on different phases of carcinogenesis.

Palut D, Kopec-Szlezak J, Kostka G. [**Significance of non-genotoxic chemical compounds in hepatocarcinogenesis (including own studies of early markers)**]. Farm Pol 1996;52(10):449-61. (Pol)

Pan YH, Reed GA. **Metabolic and genotoxic interactions of 2-aminofluorene and 2,4-diaminotoluene**. Toxicol Lett 1997;91(1):73-82.
We have reported previously that the rodent carcinogen 2,4-diaminotoluene (2,4-DAT) is not activated as a mutagen to the standard Ames S. typhimurium tester strains when oxidized by prostaglandin H synthase (PHS). 2,4-DAT does, however, enhance the bacterial mutagenicity of the potent mutagen 2-aminofluorene (2-AF) when both compounds are incubated with the PHS activating system. Enhancement of activation of 2-AF would provide a plausible mechanism for the observed co-mutagenicity of 2,4-DAT. Co-incubation with 100 microM 2,4-DAT, however, inhibited the total metabolism of 25 microM 2-AF by 60% in both the PHS/H2O2 system and PHS/ arachidonic acid system. The inhibition included a 75% decrease in the formation of water-soluble and protein-bound metabolites and about a 35% decrease in production of the peroxidative metabolites 2-nitrofluorene (NF) and 2-aminodifluorenylamine (ADFA). Azofluorene (AzF) production was the most sensitive to the effects of 2,4-DAT,

exhibiting an 80% decrease in both PHS-catalyzed systems. No new 2-AF derived products were observed in the presence of 2,4-DAT. This pronounced inhibition of 2-AF metabolism by 2,4-DAT also was observed in incubations of the aromatic amines with PHS in the presence of S. typhimurium strain TA98. Bacterial N-acetylation of 2-AF did not appear to be an important reaction in any of these incubations. 2,4-DAT not only inhibited 2-AF metabolism by PHS, but also decreased the level of 2-AF covalent binding to the bacterial DNA by as much as 81%. This stands in sharp contrast to the enhancement of the mutagenicity of 2-AF elicited by 2,4-DAT in these same incubations. This clear dissociation between the extent of peroxidative activation, and resultant covalent modification of bacterial DNA, by 2-AF and the subsequent mutagenic response indicates that a metabolic interaction is not involved in the co-mutagenicity of 2,4-DAT.

Park KK, Sohn Y, Liem A, Kim HJ, Stewart BC, Miller JA. **The electrophilic, mutagenic and tumorigenic activities of phenyl and 4-nitrophenyl vinyl ethers and their epoxide metabolites**. Carcinogenesis 1997;18 (2):431-7.

Purdy R. **A mechanism-mediated model for carcinogenicity: model content and prediction of the outcome of rodent carcinogenicity bioassays currently being conducted on 25 organic chemicals**. Environ Health Perspect 1996;104 (Suppl 5):1085-94.
A hierarchical model consisting of quantitative structure-activity relationships based mainly on chemical reactivity was developed to predict the carcinogenicity of organic chemicals to rodents. The model is comprised of quantitative structure-activity relationships, QSARs based on hypothesized mechanisms of action, metabolism, and partitioning. Predictors included octanol/water partition coefficient, molecular size, atomic partial charge, bond angle strain, atomic acceptor delocalizibility, atomic radical superdelocalizibility, the lowest unoccupied molecular orbital (LUMO) energy of hypothesized intermediate nitrenium ion of primary aromatic amines, difference in charge of ionized and unionized carbon-chlorine bonds, substituent size and pattern on polynuclear aromatic hydrocarbons, the distance between lone electron pairs over a rigid structure, and the presence of functionalities such as nitroso and hydrazine. The model correctly classified 96% of the carcinogens in the training set of 306 chemicals, and 90% of the carcinogens in the test set of 301 chemicals. The test set by chance contained 84% of the positive thio-containing chemicals. A QSAR for these chemicals was developed. This posttest set modified model correctly predicted 94% of the carcinogens in the test set. This model was used to predict the carcinogenicity of the 25 organic chemicals the U.S. National Toxicology Program was testing at the writing of this article.

Seiler F, Rehn B, Kamino K, Emuro M, Bruch J. **Significance of cell type specific formation and elimination of DNA-adducts in respiratory tissues of hamster and rat induced by alkylating chemical carcinogens**. Exp Toxicol Pathol 1996;48(6):544-7.

Shu L, Hollenberg PF. **Alkylation of cellular macromolecules and target specificity of carcinogenic nitrosodialkylamines: metabolic activation by cytochromes P450 2B1 and 2E1**. Carcinogenesis 1997;18 (4):801-10.
The alkylation of DNA, RNA and protein by labeled metabolites of [alpha-14C]nitrosodimethylamine (NDMA), [alpha-14C]nitrosodipropylamine (NDPA) and [alpha-14C]nitrosodibutylamine (NDBA) was determined as a measure of the metabolic activation of these nitrosamine carcinogens in vitro using microsomes prepared from freshly isolated rat hepatocytes as well as in intact cells using primary cultured rat hepatocytes. The abilities of these nitrosodialkylamines to alkylate cellular macromolecules were significantly affected by pretreatment of rats with inducers of cytochrome P450 and were related to the specific activities of cytochrome P450 2B1 or 2E1 in rat hepatocytes. Pretreatment of rats with phenobarbital (PB) substantially increased the catalytic activity of pentoxyresorufin (PR) O-dependentylase, an activity catalyzed by cytochrome P450 2B1, in rat hepatocytes. The increase in the PR O-dependentylase activity was associated with a significant increase in the alkylation of DNA or RNA by NDPA, and in alkylation by NDBA, particularly of proteins. However, induction of cytochrome P450 2B1 resulted in a significant decrease in alkylation of cellular macromolecules by NDMA in all cases. In contrast, enhancement of the catalytic activity of the p-nitrophenol (pNP) hydroxylase (P450 2E1) due to pretreatment of rats

with pyridine (PYR) resulted in a significant increase in the alkylation of cellular DNA by NDMA. The induction of cytochrome P450 2E1 also increased the alkylation of DNA and RNA by NDPA, but to a lesser extent. Inhibition studies using the chemical inhibitors orphenadrine (OP) and diethyldithiocarbamate (DDC), which are specific for cytochromes P450 2B1 and 2E1, respectively, indicated that cytochrome P450 2B1 was not involved in the metabolic activation of NDMA and that cytochrome P450 2E1 was not responsible for the bioactivation of NDBA. The results presented here demonstrate the substrate specificity and important role of cytochromes P450 2B1 and 2E1 in the bioactivation of nitrosodialkylamines, and suggest that multiple mechanisms may be involved in carcinogenesis induced by nitrosodialkylamines.

Sorensen IK, Kristiansen E, Mortensen A, Van Kranen H, Van Kreijl C, Fodde R, Thorgeirsson SS. **Short-term carcinogenicity testing of a potent murine intestinal mutagen, 2-amino-1-methyl-6-phenylimidazo(4,5-b) pyridine (PhIP), in Apc1638N transgenic mice**. Carcinogenesis 1997;18(4):777-81.
Transgenic Apc1638N mice, heterozygous for a targeted frameshift mutation at codon 1638 of the endogenous adenomatous polyposis coli (APC) gene, are predisposed to develop multiple adenomas and adenocarcinomas along the intestinal tract and to a number of extra-intestinal lesions including, among others, mammary tumors. We have studied these mice in a short-term carcinogenicity test with 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP), a potent murine small intestinal mutagen and lymphomagen. Upon dietary administration of 0.03% PhIP in a short-term (6 months) study, a significantly increased number of small intestinal tumors as well as an increased number of aberrant crypt foci (ACF) were observed in male Apc+/Apc1638N mice compared with untreated transgenic mice. No differences in intestinal and mammary tumor multiplicity were observed between treated and control Apc+/Apc1638N females.

Tennant RW, Spalding J. **Predictions for the outcome of rodent carcinogenicity bioassays: identification of trans-species carcinogens and noncarcinogens**. Environ Health Perspect 1996;104(Suppl 5):1095-100.
Thirty chemicals or substances currently undergoing long-term carcinogenicity bioassays in rodents have been used in a project to further evaluate methods and information that may have the capability of predicting potential carcinogens. In our predictions the principal information used includes structural alerts and in vitro test results for Salmonella mutagenicity, relative subchronic toxicity, and the sites and types of pathology found in subchronic (90-day) studies. This group of chemicals differs significantly from those used previously to evaluate predictive methods in that 23 of 30 are defined as nonmutagenic by conventional criteria. The goal of this predictive effort is to identify categorically the chemicals that have the capacity to induce cancers in both rats and mice (trans-species carcinogens) and those that are not carcinogenic in either rats or mice. Chemicals that show properties that may be associated with tumor induction in either species, i.e., species-specific cancers, are categorized as being of uncertain predictability. This category includes chemicals believed to have limited carcinogenic potential that is manifested principally as a consequence of the genetic background of the test strain of inbred rodent.

Van Oosterhout JP, Van Der Laan JW, De Waal EJ, Olejniczak K, Hilgenfeld M, Schmidt V, Bass R. **The utility of two rodent species in carcinogenic risk assessment of pharmaceuticals in Europe**. Regul Toxicol Pharmacol 1997;25(1):6-17.
For the past 20-30 years, lifespan carcinogenicity studies for pharmaceuticals have been required to be carried out in two rodent species. Due to scientific progress, the necessity/justification of lifespan studies in two species for the assessment of carcinogenic risk of pharmaceuticals is currently under discussion. A study in one species (either rat or mouse) might suffice. To appraise the need for a study in a second species, a database was compiled of all pharmaceuticals tested for carcinogenicity for which a marketing authorization was applied for in Germany and The Netherlands since 1980. The incidence of treatment-related tumor findings was determined in either rat or mouse or in both. Tumor findings occurred for nearly 50% of all compounds, with the rat being more sensitive than the mouse. Specific attention was given to the question whether tumor findings in mice ever caused the regulatory authorities to refuse registration, to restrict the proposed therapeutic indication of a pharmaceutical, or to apply a cautionary label. It was found that no tumor findings in mice alone ever led to such a regulatory action. In addition, whether mouse studies had been important in interpreting the results of rat studies was determined. A negative mouse study (no tumors found) was rarely used to declare the rat findings irrelevant to humans. A mechanistic explanation was used

as a much more important argument in the assessment of tumor findings in rats. In case of transspecies findings, the target organs were the usual ones, such as lung and liver, or the tumors occurred as a result of an exaggerated pharmacodynamic action expected from the pharmacology of the compound. The results of the database thus question the need of maintaining the requirement of rodent carcinogenicity studies in two species.

Vineis P. **Molecular epidemiology: low-dose carcinogens and genetic susceptibility**. Int J Cancer 1997;71(1):1-3.

Whiteley LO, Hudson L Jr, Pretlow TP. **Aberrant crypt foci in the colonic mucosa of rats treated with a genotoxic and nongenotoxic colon carcinogen**. Toxicol Pathol 1996;24(6):681-9.
Aberrant crypt foci (ACFs) are putative preneoplastic lesions in the colonic mucosa identified by examining methylene blue-stained whole mounts of colon. ACFs have been previously described in rats treated with genotoxic colon carcinogens. This study determined whether or not a nongenotoxic colon carcinogen could induce ACFs and compared the morphology of these ACFs with those induced by a genotoxic colon carcinogen. Six-wk-old Fischer-344 rats were administered dextran sulfate (DSS, nongenotoxin) in the drinking water or azoxymethane (AOM, genotoxin) by single subcutaneous injection. Rats were sacrificed at 9 and 14 wk after study initiation. Colons were fixed and stained with methylene blue, and the mucosal surface of transilluminated whole mounts was examined with a microscope. The number of ACFs and number of crypts per focus (multiplicity) were recorded. Representative ACFs were processed into glycol methacrylate for hexosaminidase enzyme histochemistry and sections of the remaining colon containing ACFs were embedded in paraffin for morphologic evaluation. In whole mounts, ACFs from AOM- and DSS-treated rats had elongated slit-to-oval-shaped lumens surrounded by a thickened and intensely stained epithelium. DSS-induced aberrant crypts differed from those induced by AOM in that they were frequently larger, tended not to form discrete foci circumscribed by normal crypts, and were located adjacent to ulcers. Total ACFs and large foci (4 or more crypts/focus) were significantly more numerous in AOM-treated rats at both time points. Histologically, DSS-induced ACFs had segmental to diffuse loss of hexosaminidase activity, mucin depletion to increased prominence of goblet cells, and marked distortion of crypt architecture. AOM-induced ACFs had diffuse loss of hexosaminidase activity, variable depletion of mucin, and less distortion of crypt architecture.

Yamaguchi R, Hirano T, Asami S, Sugita A, Kasai H. **Increase in the 8-hydroxyguanine repair activity in the rat kidney after the administration of a renal carcinogen, ferric nitrilotriacetate**. Environ Health Perspect 1996;104 (3):651-3.
One type of oxidative DNA damage, 8-hydroxyguanine (8-OH-Gua), is known to increase in rat kidney DNA after the administration of a renal carcinogen, ferric nitrilotriacetate (Fe-NTA). To determine the involvement of oxygen radicals in Fe-NTA carcinogenesis, we examined whether the 8-OH-Gua repair enzymes are induced in the rat kidney after Fe-NTA administration, in addition to our analysis of the 8-OH-Gua levels in the DNA, because the 8-OH-Gua repair activity is known to be induced in mammalian cells by oxidative stress due to ionizing radiation. The 8-OH-Gua repair enzyme activity was determined with an endonuclease assay using a 22-mer double strand DNA, which contains 8-OH-Gua at a specific position. A significant increase in the 8-OH-Gua repair activity was observed in the rat kidney after a single intraperitoneal injection of Fe-NTA ($p < 0.01$). This is the first report on induction of the repair activity for 8-OH-GUA after treatment with a chemical carcinogen. This assay will be useful for evaluating the carcinogenicity of oxygen radical-forming chemicals..

Yoshikawa K. **Anomalous nonidentity between Salmonella genotoxicants and rodent carcinogens and genotoxic noncarcinogens**. Environ Health Perspect 1996;104(1):40-6.
BIOSIS COPYRIGHT: BIOL ABS. According to current data, the capacity of cause non-programmed or unscheduled cell proliferation in target tissues, a common characteristic of chemical carcinogens, may play a more important role in the development of tumors than does genotoxicity. This paper provides strong support for the validity of this conclusion. Ames-negative nongenotoxicants may be considered to be carcinogenic primarily because of their ability to induce cell proliferation in animal tissues and organs. In addition, such nongenotoxic

carcinogens may also provide latent nad modest DNA (equivocal) modifications that never lead to Ames-positive events. Conversely, noncarcinogenesis by Ames-positive agents is likely to be linked to a lack of stimulation of cell division. Nongenotoxic and genotoxic carcinogens rely on both cell proliferation and equivocal DNA modification for their full carcinogenicity. Such equivocal DNA modifications do not appear to be formed by tumor promoters. The role of cell proliferation may provide a favorable milieu for the occurrence of genetic instability, give rise to selective apoptosis-resistant abnormal cells, and then affect clonal expansion of these cells. Therefore, understanding the influence of nongenotoxic and genotoxic carcinogens on cell proliferation capability is a key point in determining the mechanisms of chemical carcinogenesis. Considering the contradictory and common features of genotoxicants and carcinogens, early detection of nonprogrammed cell proliferation is the most effective approach to predict human and rodent carcinogenicity.

Zhang YP, Sussman N, Macina OT, Rosenkranz HS, Klopman G. **Prediction of the carcinogenicity of a second group of organic chemicals undergoing carcinogenicity testing**. Environ Health Perspect 1996;104(Suppl 5):1045-50.
Twenty-four organic compounds currently undergoing testing within cancer bioassays under the aegis of the U.S. National Toxicology Program (NTP) were submitted to the computer automated structure evaluation (CASE) and multiple computer automated structure evaluation (MULTICASE) system for predictions of activity. Individual predictions resulting from the NTP combined rodent, NTP mouse, Carcinogenic Potency Database (CPDB) combined rodent, and CPDB mouse databases were combined using Bayes' theorem to yield an overall probability of rodent carcinogenicity. Based upon an arbitrary probability cut-off of 0.50, nine compounds were predicted to be rodent carcinogens. The predicted carcinogens are chloroprene, 1-chloro-2-propanol, codeine, emodin, furfuryl alcohol, isobutyraldehyde, primaclone, sodium xylenesulfonate, and t-butylhydroquinone.

CYTOTOXICITY

Atkins TW, Tighe BJ. **Preliminary in vitro cytotoxicity screening of a bead-formed macroporous hydrophilic polymer matrix**. J Biomater Sci Polym Ed 1996;7(9):759-68.
A prescreen of the in vitro cytotoxicity of both the primary fabrication components and potential leachables from a bead-formed macroporous poly(2-hydroxyethyl methacrylate), (pHEMA) matrix has been carried out using INVITTOX Neutral red and Kenacid blue R dye binding methods. Of the eluants obtained from 24, 48, and 72-h incubated beads, only the 72-h eluant produced a greater than 20% (ID20) inhibition of 3T3-L1 cell proliferation with values of 20.98 +/- 2.33% and 21.41 +/- 1.37% inhibition for the Neutral red and Kenacid blue R binding methods, respectively. ID50 values for the fabrication components obtained using the Kenacid blue R method were generally higher than those obtained by the Neutral red assay, although the ranking of the chemicals in terms of their relative cytotoxicities was identical by both methods, i.e. ethylene glycol dimethacrylate > uranyl nitrate > purified HEMA > n-hexane > ethylene glycol (mmol 1(-1)). Whilst extended washing of finished PHEMA beads in water will reduce their acute in vitro cytotoxicity, this will only be achieved with some loss of previously encapsulated water soluble macromolecules.

Bertheussen K, Yousef MI, Figenschau Y. **A new sensitive cell culture test for the assessment of pesticide toxicity**. J Environ Sci Health B 1997;32(2):195-211.
A new, simple and sensitive cell culture test for the determination of cytotoxicity of pesticides is described. This test is based on assessment of the growth inhibitory effect of determination of

cytotoxicity of pesticides is described. This test is based on assessment of the growth inhibitory effect of pesticides on the rapidly growing mouse hybridoma cell line 1E6 cultivated in a defined serum-free medium (RPMI-SR3). In addition the cytotoxicity in serum-containing medium was investigated. The results showed that the sensitivity of 1E6 cell towards the toxicity of the pesticides; carbofuran, cypermethrin, lindane, glyphosate and 2,4-D was considerably higher in the serum-free medium than in serum-containing medium. Also IC50 values of these pesticides were compared with LD50 values obtained from the literature. The ratio between the in vitro IC50 and the in vivo LD50 showed that the present cell culture test determine the toxicity of low levels of pesticides with much higher sensitivity than tests based on using animals.

Brosin A, Wolf V, Mattheus A, Heise H. **Use of XTT-assay to assess the cytotoxicity of different surfactants and metal salts in human keratinocytes (HaCaT): a feasible method for in vitro testing of skin irritants**. Acta Derm Venereol 1997;77(1):26-8.
BIOSIS COPYRIGHT: BIOL ABS. Because of the increasing need of reliable skin irritation tests and in order to reduce the number of animal experiments, in vitro alternatives have to be developed. We studied four surfactants and five metal salts for their cytotoxic potency in HaCaT cells, a spontaneously immortalized human keratinocyte fine. The endpoint used to assess cellular viability was metabolization of the tetrazolium salt XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-((phenylamino)carbony- l)-2H-tetrazolium hydroxide). The tested substances revealed a significant rank order of their cytotoxicity at an exposure time of 24 h. It was 1) benzalkonium chloride, 2) sodium lauryl sulphate, and 3) Tween 20 (polyoxyethylene sorbitanmonolaurate) and Tween 80 (polyoxyethylene sorbitanmonooleate), for the surfactants; and 1) potassium bichromate, 2) copper sulphate, 3) cobalt chloride and palladium chloride, and 4) nickel sulphate, for the metal salts. There is an excellent correlation to the rank order of their known irritative potency in vivo. Being practicable and effective, the presented XTT-assay on HaCaT cells would be well suitable for an initial orientating screening of substances, subsequently followed by irritation tests directly in humans.

Figenschau Y, Yousef MI, Sveinbjornsson B, Bertheussen K. **A sensitive serum-free colorimetric assay for the detection of cytotoxic effects of pesticides**. J Environ Sci Health B 1997;32(2):177-94.
A new method of cell quantification in the Hybritest bioassay is described. This test is based on culturing the hybridoma cell line 1E6 in the serum-free medium RPMI-SR3 and determination of cytotoxicity by growth inhibition. By employing a tetrazolium salt (MTT), the cell number were quantified colorimetrically using an ELISA reader. This assay carried out in microtiter plates saved time, labour and expenses. It was demonstrated that the sensitivity of 1E6 to pesticide toxicity (glyphosate and cypermethrine) was considerably higher in serum-free medium than in serum-containing medium. Furthermore, it is suggested that this assay may represent an alternative to the use of living animals in toxicological experiments.

Giavaresi G, Torricelli P, Fini M, Giardino R. **Pericellular pO2 as an alternative method to test cytotoxicity**. Artif Cells Blood Substit Immobil Biotechnol 1996;24(6):579-86.
Pericellular pO2 (ppcO2) was compared with the release of cytoplasmatic enzyme lactate dehydrogenase (LDH) and mitochondrial metabolic function (tetrazolium salt reduction, MTT) as an alternative method to evaluate cytotoxicity. L-929 cells were seeded and incubated with 3 different

control materials to test their cytotoxicity effects by these methods. High density polyethylene and copper were respectively used as negative and positive controls, while a 0.9% NaCl solution was as reagent control and extraction vehicle. PpcO2 was measured by a rod polarographic Clark-type probe connected to Licox pO2 computer (GMS mbH, Kiel, Germany). One-way ANOVA test showed significant differences among groups regarding every methods ($p < 0.0005$). The comparison of the mean variation coefficients of three methods showed no significant differences. In addition, a significant correlation was found between PPCO2 and MTT ($R = 0.621$; $P < 0.001$) as well as PPCO2 AND LDH DATA ($R = 0.474$; $P < 0.001$). In conclustion, PPCO2 may be considered a reliable, safely and alternative method to test cytotoxicity.

Hilger I, Aufderheide M, Knebel JW, Fuchs S, Emura M. **Sensitivity of a hamster lung cell line to direct and indirect acting carcinogens**. Exp Toxicol Pathol 1996;48(6):532-4.
Cytotoxicity of benzo(a)pyrene (B(a)P), 7,12-dimethylbenz(a)anthracene (DMBA), aflatoxin B1 (AB1), and N-methyl-N'-nitro-N- nitrosoguanidine (MNNG) was estimated in vitro by using a hamster lung cell line. The studies were conducted to assess the usefulness of an organ-specific cell culture system for demonstrating differences in the cytotoxic potency of diverse chemical carcinogens. Cytotoxicity was determined by using the succinate dehydrogenase assay (MTT assay) after different incubation times and concentrations with the corresponding carcinogens. The effective concentration EC50 as well as the slope of the regression line were used as parameters for the biological effects. The results from these studies indicate a clear dose-dependent reaction after incubation of the cells with aflatoxin B1 (EC50: 2.3 microM) and MNNG (EC50: 4.0 microM). For the polycyclic hydrocarbons benzo(a)pyrene and DMBA, a dose-independent reaction was found. These results indicate that consideration of the EC50 values only might not be sufficient to characterize differences in the cytotoxic activity of different substances. Chemicals can lead to equal values in the EC50, but cells can differ significantly in their biological sensitivity, meaning that the extent of reduction in cell proliferation depends on the chemical used. By considering the two above-mentioned parameters, a ranking for the analyzed substances will be possible in the following way: AB1, MNNG, DMBA and B(a)P. Taken together, our experiments show that it is possible to reveal differences in the cytotoxic potency of chemicals by using in vitro methods.

Tubaro A, Florio C, Luxich E, Vertua R, Della Loggia R, Yasumoto T. **Suitability of the MTT-based cytotoxicity assay to detect okadaic acid contamination of mussels**. Toxicon 1996;34(9):965-74.
The suitability of a cytotoxicity assay based on the MTT colorimetric method has been evaluated for the detection of okadaic acid in mussels. On KB cells, okadaic acid exhibited a dose-dependent cytotoxic effect, the IC50 being inversely related to the exposure time (IC50 = 6.3 ng/ml, 4.0 ng/ml and 1.1 ng/ml after 24, 48 and 72 hr of contact, respectively). Using a contact time of 24 hr, the MTT cytotoxicity assay is suitable for revealing okadaic acid concentrations in mussel samples as low as 50 ng/g of digestive glands, with a sensitivity higher than that of the commercially available kits for enzyme-linked immunosorbent assay (ELISA). In the okadaic acid concentration range from 50 to 1500 ng/g of digestive glands the MTT cytotoxicity assay showed satisfactory accuracy and reproducibility. A high degree of correlation was found between the okadaic acid content of 16 naturally contaminated samples measured by the MTT cytotoxicity assay and by an ELISA.

DERMAL TOXICITY

Abduliah D, Ping QN, Liu GJ. **Enhancing effect of essential oils on the penetration of 5-fluorouracil through rat skin**. Acta Pharm Sinica 1996 Mar;31:214-21.
IPA COPYRIGHT: ASHP The effects of eucalyptus oil, peppermint oil, and turpentine (turpentine oil) on the penetration of fluorouracil (5-fluorouracil) using excised rat skin were evaluated and compared with results from laurocapram (azone). The oils enhanced penetration of fluorouracil, but less than laurocapram. Eucalyptus oil was the most active oil. All oils increased partition coefficients and the diffusion coefficient values were comparatively higher.

Ademola JI, Maibach HI. **Cutaneous metabolism and penetration of methoxypsoralen, betamethasone 17-valerate, retinoic acid, nitroglycerin and theophylline**. Curr Probl Dermatol 1995;22:201-13.

Bando H, Sahashi M, Mohri S, Yamashita F, Takakura Y, Hashida M. **In vivo skin penetration enhancement of acyclovir by theoretical design of prodrug-enhancer combination**. Int J Pharm 1996;145(1-2):103-13.
CBAC COPYRIGHT: CHEM ABS The effectiveness of prodrug-enhancer combination under in vivo skin penetration enhancement was studied using acyclovir and its lipophilic prodrugs, i.e. acyclovir valerate, isovalerate and pivalate together with an enhancer, 1-geranylazacycloheptan-2-one (GACH). Under in vivo penetration expt. with rat skin, the authors estd. absorption amts. of both prodrug and metabolized acyclovir resp. from the excretion amt. by employing a deconvolution method. In the absence of GACH, the total amt. of acyclovir absorbed at the end of 4 h expt., after application of prodrug in the form of aq. soln., was about twice than that obtained after administration of acyclovir itself. On the other hand, GACH showed slight absorption enhancement for acyclovir but demonstrated drastic enhancement effect on its prodrugs, esp. valerate. Also, this enhancement effect was more remarkable under in vivo condition than in vitro one. Indeed, to elucidate the differences in enhancement effect among three prodrugs, the authors carried out some simulations to clarify the relationship among enhancement effect, lipophilicity of prodrugs and enzymic hydrolysis rate consts. From the results of simulations, it was obviously noticed that metab. only exerted an important effect on skin penetration of these prodrugs when applied with GACH simultaneously. Furthermore, according to this model anal. under in vivo condition, the authors came to understand that GACH significantly decreased the enzymic activity in skin.

Bando H, Sahashi M, Takagi T, Yamashita F, Hashida M, et al . **Analysis of in vitro skin penetration of acyclovir prodrugs based on a diffusion model with a metabolic process**. Int J Pharm 1996 Jun 17;135:91-102.
IPA COPYRIGHT: ASHP The penetration of 7 acyclovir prodrugs through the rat skin with or without an enhancer, 1-geranylazacycloheptan-2-one (GACH), was analyzed based on a newly developed 2-layer skin diffusion model with polar and nonpolar routes in the stratum corneum including metabolic process. Laplace-transformed equations for prodrug and regenerated acyclovir were derived from Fick's second law assuming first order hydrolysis and were fitted to the experimental data. Under the condition without GACH treatment, more lipophilic prodrugs gave higher partition parameters in the nonpolar route. The enzymatic hydrolysis rate constants estimated by model analysis of the penetration experiment were basically similar in rank order to those obtained using skin homogenate. Concerning the effects of GACH, the estimated partition parameters of prodrugs in the nonpolar route increased with an increase in pretreatment dose of GACH, but their diffusivities were little affected being in good agreement with the theoretical prediction. In addition, GACH significantly decreased thetheoretical prediction. In addition, GACH significantly decreased the enzymatic hydrolysis rate constants of all prodrugs in the skin.

Bangha E, Maibach HI, Elsner P. **Toxicology of topical local anesthetics**. Skin Pharmacol 1996;9(6):376-80.
Topical anesthesia of the skin, nowadays performed for various indications from pruritus over postherpetic neuralgia to minor surgery, has been under investigation for more than 30 years. Due to low water solubility, the active base form of most of the local anesthetics on the market is poorly absorbed through the skin. Hence, the most challenging target was to develop galenic preparations which provide a good skin penetration in order to reach the

dermal nerve endings and thereby lead to sufficient local anesthesia. On the other hand good skin penetration also results in a distribution of the drug in the circulation. Since local anesthetic agents are known to have an impact on the heart and central nervous system, unwanted side effects following topical application onto the skin are worth discussing. This article reviews the current topical local anesthetics with particular accent on their pharmacological and toxicological data.

Barratt MD. **Quantitative structure-activity relationships for skin permeability**. Toxicol In Vitro 1995;9(1):27-37.
An analysis of quantitative structure activity relationships (QSARs) associated with the skin penetrability of chemicals was performed. Published data on the in-vitro permeability coefficients (PCs) of 97 organic compounds were utilized. The compounds were divided into the following classes: steroids, other pharmacologically active compounds (OPACs), and small organic molecular (SOM) compounds. Possible associations between the logarithm of the PCs (logPCs) of the compounds and their octanol/water partition coefficients (logPs), molecular volume (MV), or melting point (mp) were examined using multiple regression techniques. An initial regression of the logPCs against logP and MV produced a straight line with a correlation coefficient of 0.711. Including mp in the analysis improved the fit; correlation coefficient 0.765. Since the experimental logPCs of many of the hydrocortisone esters were below the line of best fit, suggesting that these molecules might have a correlation independent of the main group, a principal components analysis of the dataset was performed using logPC, logP, MV, and mp as the independent variables. This confirmed the validity of dividing the dataset into steroid, OPAC, and SOM compounds. Additional multiple regression analyses of the three classes of molecules indicated that their logPCs were well correlated with the other descriptors for steroid and SOM compounds. The correlation of the OPACs was poor. A separate analysis of the hydrocortisones in the steroid group produced a correlation coefficient of 0.885. An analysis of the steroid and SOM compounds (72 compounds total), produced a correlation coefficient of 0.838. The hydrocortisone logPCs were again below the line of best fit. Repeating the analysis, but excluding the hydrocortisones (60 compounds total), produced a correlation coefficient of 0.903. The author concludes that these analyses show that a QSAR relating logPC to logP, MV, and mp should be capable of accurately predicting the ability of a variety of organic compounds to penetrate human skin in-vitro.

Barratt MD. **Quantitative structure-activity relationships (QSARs) for skin corrosivity of organic acids, bases, and phenols: principal components and neural network analysis of extended datasets**. Toxicol In Vitro 1996;10(1):85-94.
An analysis of possible quantitative structure activity relationships (QSARs) associated with the skin corrosivity potential of acids, bases, and phenols was performed. Published data on skin irritancy tests of 50 inorganic and organic acids, 40 organic bases, and 33 phenolic compounds were used.

Bast GE, Kampffmeyer HG. **No effect of albumin on the dermal absorption rate of hydrocortisone 21-butyrate, permethrin or diflunisal in the isolated, single-pass perfused rabbit ear**. Skin Pharmacol 1996;9(5):327-33.
CBAC COPYRIGHT: CHEM ABS The penetration rate of hydrocortisone (I), permethrin, and diflunisal into skin or the effusate was examd. enhanced by the addn. of hetastarch (HES) or bovine serum albumin (BSA) to buffer soln. for single-pass perfusion. Following cutaneous ester hydrolysis, the appearance rate of I was 4 pmol/min per cm2 in HES and BSA contg. buffer. BSA in the perfusion fluid may not enhance the appearance rate of xenobiotics in the effluent following dermal application when the distribution coeff. n-octanol/water is .gtoreq. 2,000 or when the xenobiotic is ionized at physiol. pH.

Baynes RE, Brownie C, Freeman H, Riviere JE. **In vitro percutaneous absorption of benzidine in complex mechanistically defined chemical mixtures**. Toxicol Appl Pharmacol 1996;141(2):497-506.
BIOSIS COPYRIGHT: BIOL ABS. Little work has been done on the topical absorption of the bladder carcinogen benzidine. Since humans are more likely to be exposed to chemical mixtures than to a single chemical, a program was developed in these laboratories to examine the cumulative effect of complex mixtures on percutaneous absorption of important toxicants such as benzidine. In this investigation, a mixture is defined as a physical combination consisting of a marker chemical and several other chemicals, each of which can have independent and/

or synergistic effects on dermal penetration and absorption of the marker chemical. Ten mixtures, consisting of a marker chemical (benzidine, B), a solvent (acetone, A or DMSO, D), a surfactant (0 or 10% sodium lauryl sulfate, SL), a vasodilator (0 or 180 mug methyl nicotinate, M), and a reducing agent (0 or 2% SnCl2, s) were employed in this study. Isolated perfused porcine skin flaps (IPPSFs), which have proven to be a suitable in vitro model for assessing dermal absorption and toxicity, and flowthrough diffusion cell systems were utilized. The extent of benzidine absorption in skin sections dosed with either B + A (0.94% dose) or B + D (1.01% dose) was similar to that when IPPSFs were dosed with either B + A (0.54% dose) or B + D (1.31% dose). However, flux vs time profiles were different when the two in vitro methods were compared. For mixtures containing (1) DMSO only or acetone only or (2) solvents containing SL + M, benzidine absorption was enhanced when compared with other mixtures. Compared to acetone, DMSO appears to enhance dermal penetration of benzidine in most of the mixtures. Compared to other mixtures evaluated, SnCl2 inhibited benzidine absorption irrespective of solvent present. SnCl2 also appears to inhibit benzidine penetration in DMSO mixtures containing SL only, but not in acetone mixtures. It is proposed that chemical-chemical interactions between benzidine and SnCl2 may be inhibiting benzidine absorption and chemical-biological interactions between M + SL and skin may be enhancing benzidine absorption. Across all mixtures, maximum observed benzidine absorption was almost 3% of the topical dose over 8 hr, but maximum penetration was 22% over the same time period which would suggest a potential for greater systemic exposure over longer time frames. This work underscores the need to study potentially toxic chemicals in mixture exposure scenarios since the interactions observed would confound risk assessment based on single chemical data.

Bernard E, Dubois J, Wepierre J. **Importance of sebaceous glands in cutaneous penetration of an antiandrogen: target effect of liposomes**. J Pharm Sci 1997;86(5):573-8.
CBAC COPYRIGHT: CHEM ABS The significance of the sebaceous gland pathway in the cutaneous permeation of an antiandrogen, 4-[3-(4-hydroxybutyl)-4,4-dimethyl-2,5-dioxo-1-imidazolidinyl]-2-- (trifluoromethyl)benzonitrile (RU 58841), was studied with normal hairless rat skin and an induced scar hairless rat skin without sebaceous glands. RU 58841 was dissolved in an alc. soln. and encapsulated in liposomes for comparison. After 24 h, the cumulative percentage of RU 58841 absorbed in vitro was 3-4-fold higher in the normal skin than in the scar skin; in the case of liposomes, the accumulation of the drug in the normal dermis was significantly higher than in the scar one. In the in vivo cutaneous distribution, the epidermis and dermis of the normal skin contained higher amts. of RU 58841 than the scar skin (ninefold with the soln. and 16-fold with liposomes). An autoradiog. study showed that with the soln., the drug was mainly localized in the stratum corneum/epidermis, and with the liposomes, the drug was mainly localized in the sebaceous glands. We concluded that the sebaceous glands constituted the main pathway for RU 58841. The alc. soln. encouraged the localization of the drug into the stratum corneum, whereas liposomes targeted the sebaceous glands.

Bernkop-Schnurch A, Valenta C, Gatterwe V. **In vitro skin permeation studies of the antibiotic nisin**. Eur J Pharm Biopharm 1996;42(5):336-9.
IPA COPYRIGHT: ASHP Passive and iontophoretic skin permeation kinetics of nisin (nisin A) were studied in vitro using shaved rat skin. Nisin was quantified using a Franz diffusion cell in combination with an enzyme linked immunosorbent assay based on polyclonal antibodies against nisin. The passive diffusion over the first 4 h of 1 mg/ml of nisin was < 100 PG/SQ CM. The cumulative amount of nisin pereation during 6 h of iontophoretic transport (0.5 MA/SQ CM) was 112 NG/SQ CM, which corresponded to abput 0.015% permeation. The passive diffusion results suggested that the detergent like properties of nisin that might function as penetration enhancers did not have an accelerating influence on its penetration.

Beskitt JL, Sun JD. **In vitro skin penetration characteristics of ethanol in the rabbit, mouse, rat, and human**. J Toxicol Cutan Ocular Toxicol 1997;16(1):61-75.
BIOSIS COPYRIGHT: BIOL ABS. The cutaneous penetration of chemicals can be measured by in vitro techniques using a wide variety of animal and human skin samples. In contrast to dermal penetration studies using whole animals, in vitro techniques allow the direct measurement of chemical penetration, which can be used to help predict the absorbed dose and rate of absorption in whole animals and across species. Thus, in vitro methods may provide results that allow better extrapolations of in vivo animal studies to human exposure scenarios, and more

accurate assessments of the potential human health risk following dermal exposure to chemicals. When conducting in vitro skin penetration studies, it is critical to know the condition and integrity of skin samples being used. Skin samples that have been improperly handled and/or stored can yield results that either underestimate or overestimate true penetration characteristics. To avoid this, ethanol was used as a model compound to assess the quality of skin samples used for skin penetration studies. During each experimental session, the penetration characteristics of a 25% aqueous solution of (14C)ethanol were measured and the results compared with values obtained from similar control experiments conducted previously. This paper describes the compilation of ethanol penetration data collected over 6 years using full-thickness rat, mouse, rabbit, and human skin samples from over 175 experiments. In general, the results showed that the order of skin permeability for this model compound was rabbit > mouse > ratase has allowed the statistical description o the distribution of such data and, from this, has established skin penetration criteria of ethanol as a reference chemical that can be used to assess the condition and integrity of skin samples used for in vitro skin penetration studies.

Bhatia KS, Gao S, Singh J. **Effect of penetration enhancers and iontophoresis on the FT-IR spectroscopy and LHRH permeability through porcine skin**. J Control Release 1997;47(1):81-9.
CBAC COPYRIGHT: CHEM ABS The present study explores the effect of enhancer and iontophoresis on the in vitro permeability of LH releasing hormone (LHRH) through porcine epidermis and biophys. changes in the stratum corneum lipids by Fourier-transform IR (FT-IR) spectroscopy. Enhancers (e.g. ethanol, 10% oleic acid in combination with ethanol and 10% oleic acid in combination with propylene glycol) pretreatment increased the permeability coeff. of LHRH. Propylene glycol alone did not increase the permeability of LHRH through the epidermis. Iontophoresis further increased the permeability of LHRH through the enhancers (ethanol, 10% oleic acid in combination with ethanol, propylene glycol and 10% oleic acid in combination with propylene glycol) pretreated epidermis in comparison to the control (without enhancer pretreated epidermis). Iontophoresis can synergize with enhancers, such as 10% oleic acid in combination with ethanol and 10% oleic acid in combination with propylene glycol, to provide an addnl. driving force to maintain and control the target flux of LHRH. The FT-IR spectroscopic study was performed to investigate the effect of the above enhancers and iontophoresis on the biophys. changes in the stratum corneum lipids. Pretreatment of the stratum corneum with 10% oleic acid in combination with ethanol, and 10% oleic acid in combination with propylene glycol followed by iontophoresis shifted the antisym. peak position with ref. to the control (without enhancer and iontophoresis) from 2919.3 to 2927.6, and 2919.3 to 2924.2 cm-1, resp. Thus, the combination of enhancer and iontophoresis increased the av. acyl chain disorder (lipid fluidity). The synergism in enhancement of the permeability of LHRH through the epidermis by iontophoresis in combination with enhancers (10% oleic acid in combination with ethanol, and 10% oleic acid in combination with propylene glycol) may be due to greater fluidization of the stratum corneum lipids.

Boelsma E, Tanojo H, Bodde HE, Ponec M. **Assessment of the potential irritancy of oleic acid on human skin: evaluation in vitro and in vivo**. Toxicol In Vitro 1996;10(6):729-42.
BIOSIS COPYRIGHT: BIOL ABS. As skin barrier modulating compounds, fatty acids are frequently used in formulations for transdermal or topical delivery. In this study the effects of oleic acid on keratinocytes in vitro was compared with its in vivo skin irritancy in humans. Dose- and time-dependent effects of oleic acid were examined in submerged human keratinocyte cultures, in reconstructed human epidermis (RE-DED). and in excised human skin, using alterations in morphology and changes in interleukin-1alpha mRNA levels as endpoints. In vitro results were compared with responses of living human skin after topical application of oleic acid. using non-invasive bioengineering methods. Direct interaction of oleic acid and submerged keratinocyte cultures resulted in cell toxicity at very low concentrations of the fatty acid. By contrast, when oleic acid was applied topically on RE-DED or on excised skin, no alterations in morphology were observed. Modulation of stratum corneum thickness indicated a key role of the stratum corneum barrier in the control of oleic acid-induced toxicity. In agreement with these findings, no epidermal tissue damage was seen in vivo, whereas oleic acid induced a mild but clearly visible skin irritation and inflammatory cells were present in the upper dermal blood vessels. Small amounts of oleic acid induced IL-1alpha mRNA expression in submerged keratinocyte cultures, whereas in RE-DED and in excised skin, IL-1alpha mRNA levels were increased only when the concentration applied topically was at least two orders of magnitude higher. It is concluded that minute amounts of oleic acid are sufficient to cause local (i.e. inside the viable epidermis)

modulation of cytokine production. These concentrations do not affect morphology but induce skin irritation in vivo. To achieve comparable effects in the skin, much higher topical doses are needed than expected according to the locally required levels, owing to the rate-limiting transport of the fatty acid across the stratum corneum barrier.

Bonina F, Lanza M, Montenegro L, Puglisi C, Tomaino A, Trombetta D, Castelli F, Saija A. **Flavonoids as potential protective agents against photo-oxidative skin damage.** Int J Pharm 1996;145(1-2):87-94.
CBAC COPYRIGHT: CHEM ABS Flavonoids, a group of phenolic compds. widely occurring in the plant kingdom, have been reported to possess strong antioxidant activity. This preliminary study was designed to est. the potential utility of topically applied flavonoids to prevent photooxidative stress in the skin. With this aim the authors evaluated the protective effect of three flavonoids (quercetin, hesperetin and naringenin), chosen according to their structural characteristics, against UV radiation-induced peroxidn. on phosphatidylcholine (PC) vesicles as a model membrane. Furthermore 'in vitro' human skin permeation of these flavonoids was measured, given that a suitable percutaneous absorption is an essential requirement for satisfactory topically applied photoprotective agents. The flavonoids tested in this study protected efficiently PC liposomes from UV radiation-induced peroxidn., probably by scavenging oxygen free radicals generated by UV irradiations; their antilipoperoxidative activity can be classified as follows: quercetin>hesperetin>naringenin. In addn., naringenin, hesperetin and, at a very lower degree, quercetin permeated through the stratum corneum (which is the main barrier against the penetration of exogenous substances through the skin) and, so, to penetrate into deeper skin layers. Thus, topically applied flavonoids could be excellent candidates for successful employment as protective agents in certain skin diseases caused, initiated or exacerbated by sunlight irradn.

Bookout RL Jr, McDaniel CR, Quinn DW, McDougal JN. **Multilayered dermal subcompartments for modeling chemical absorption**. SAR QSAR Environ Res 1996;5(3):133-50.
Dermal penetration of chemicals and drugs is of concern to both toxicologists and pharmacologists. Environmental professionals try to limit exposure to chemicals using protective clothing and gloves or barrier creams to trap chemicals. Drug developers try to enhance penetration of chemicals through the skin for medical purposes. Both can use predictive biologically-based mathematical models to assist in understanding the processes involved. These models are especially useful when they are based on physiological and biochemical parameters which can be measured in the laboratory. Appropriately validated models based on conservation of mass, diffusion and chemical transport by flow can be predictive of human exposures. In this paper we develop two new physiologically-based pharmacokinetic (PBPK) skin models to predict blood concentrations of dibromomethane in rats after skin-only vapor exposures. These new models improve the predictions of the blood concentrations especially at the beginning of the exposures. Sensitivity analysis shows that the permeability constants followed by partition coefficients have the most impact on blood concentration predictions. With proper validation the new models could be used to improve species, dose, and duration extrapolations of chemical or drug penetration. They could also be used to investigate and predict concentrations of drugs or chemicals in the skin.

Borras-Blasco J, Lopez A, Morant MJ, Diez-Sales O, Herraez-Dominguez M. **Influence of sodium lauryl sulfate on the in vitro percutaneous absorption of compounds with different lipophilicity**. Eur J Pharm Sci 1997;5 (1):15-22.

Chou WL, Cheng CH, Yen SC, Jiang TS. **Enhanced iontophoretic transport of TRH and its impedance study**. Drug Dev Ind Pharm 1996;22(9-10):943-50.
IPA COPYRIGHT: ASHP The enhanced iontophoretic transport of the model peptide protirelin (thyrotropin releasing hormone; TRH) through an excised rabbit skin by in vitro iontophoresis is described. Results indicated that the steady state flux of protirelin in a diffusion cell was proportional to the current density. The skin impedance decreased to a low and stable value with respect to its initial skin impedance while a current was applied through the rabbit skin. Compared with passive diffusion, the iontophoresis dramatically increased the transport fluxes of protirelin, and ethyl alcohol (ethanol) pretreatment further enhanced its iontophoretic transport.

Cordero JA, Alarcon L, Escribano E, Obach R, Domenech AJ. **A comparative study of the transdermal penetration of a series of nonsteroidal antiinflammatory drugs**. J Pharm Sci 1997;86(4):503-8.
CBAC COPYRIGHT: CHEM ABS The transdermal absorption of a series of nonsteroidal antiinflammatory drugs (NSAIDs), indomethacin, ketoprofen, diclofenac, piroxicam, tenoxicam, ketorolac, and aceclofenac, was studied in vitro with human skin. The purpose of the study was to det. the permeation parameters (permeability rate const., Kp; lag time, TL, and flux, J) as measures of the intrinsic transdermal permeabilities of these drugs to predict their potential for formulation in a transdermal therapeutic system (TTS). A linear correlation was established between the intrinsic log Kp values and the intrinsic partition coeffs. (r = 0.863). Diclofenac had the highest value of in vitro transdermal penetration at .apprx.0% ionization (Kp = 3.5 cm/h) and ketoprofen had the highest flux (J = 16 mug/h. cntdot.cm2) of the NSAIDs assayed. Ketorolac would provide the plasma concns. at steady state that would be nearest to the therapeutic concn. (Ct/Css = 26). Also, considering the whole permeation profile in vitro, ketorolac would be the most suitable candidate of the series studied to be formulated as a TTS.

Diao Y, Chem Z. [**Study on penetration diffusion of diclofenac sodium films**]. Zhongguo Yaoke Daxue Xuebao 1996;27(9):531-3. (Chi)
CBAC COPYRIGHT: CHEM ABS The in vitro penetration diffusion of diclofenac sodium films was studied by using Franz equipment and animal skin. When the dosage of diclofenac sodium was 1.5 mug cm-2 in the donor compartment and 15 mL of the normal saline in receptor compartment, the accumulative amt. of the diclofenac sodium through rabbits, mice and rats skin were 70.93, 62.43 and 41.44%, resp. In the stable state, the percutaneous ratios (J) were 107.0 (rabbits), 90.26 (mice) and 64.92 11.4 mug cm-2 h (rats).

Dreher F, Walde P, Walther P, Wehrli E. **Interaction of a lecithin microemulsion gel with human stratum corneum and its effect on transdermal transport**. J Control Release 1997;45(2):131-40.
CBAC COPYRIGHT: CHEM ABS A soybean lecithin microemulsion gel was studied as a possible matrix for transdermal drug delivery. This gel is transparent and viscous, and it is composed of soybean phosphatidylcholine (lecithin), iso-Pr palmitate and a small amt. of water. In vitro percutaneous penetration studies of 2 anti-inflammatory drugs, indomethacin and diclofenac, dissolved in the gel-system resulted in steady state fluxes of about 1 mug h-1 cm-2. In order to est. the function of the gel as a potential transdermal penetration enhancing system, interaction studies with isolated human stratum corneum were performed by FTIR spectroscopy, DSC as well as low-temp. SEM. The lecithin gel, in particular iso-Pr palmitate, affects the stratum corneum lipid organization even after 1-day incubation (FTIR, DSC), whereas recent in vivo human skin irritation tests showed no significant irritancy.

Dupuis L. Multiple emulsion and urea on pig skin. **A moisturization and penetration study**. Sofw J 1996;122(10): 658,660,662-3.
CBAC COPYRIGHT: CHEM ABS A 2-step procedure W/O/W multiple emulsion was developed based on the use of colloidal silica to stabilize the oily film. Urea was incorporated in the primary and the final multiple emulsions. Tests were carried out with formulas with and without urea, in the internal or external aq. phase for multiple emulsions. The effects of urea localization were studied on pig skin ex vivo by corneometry and ATR-IR spectroscopy. The best moisturization results were obtained when urea penetrated into the stratum corneum. Urea encapsulated in the internal aq. phase reacted like urea brought on the skin with a simple W/O emulsion for penetration and moisturization. Unencapsulated urea behaved differently and did not hydrate nor penetrate.

Elliott GR, De Lange J, Bruijnzeel PL. **Epidermal barrier and depot function changes from xylene exposure in the perfused pig ear organ culture system**. J Toxicol Cutan Ocular Toxicol 1997;16(1):31-43.
BIOSIS COPYRIGHT: BIOL ABS. Factors influencing the rate of dermal permeation of xylene were studied in vitro using the perfused pig ear (PPE) model. Pig-ear skin was exposed to xylene for either 30 min, 60 min, or 240 min. Total permeation of xylene during this period was 23.2 : 5.5 mug (n = 5), 29.7 : 4.0 mug (n = 5), 28.2 : 6.4 mug (n = 6), 48.1 : 10.4 (n = 6), and 65.6 : 8.3 mug (n = 6) respectively (mean : SEM). There were no statistical differences in total permeation for exposure periods of 30 min or less and for 60 min or longer. However, the amount of xylene present in the skin was directly related to exposure time (15 min, 30 min, and 60 min). When skin was stripped

(Scotch adhesive tape, 10 times) before being exposed to xylene for 15 min, total permeation increased by about twofold to 67.9 : 15.6 mug (n = 3). In contrast, tape stripping after the 15 min exposure period resulted in a small decrease in the total amount of xylene permeating (22.3 : 3.9 mug, n = 4). Epidermal damage appeared to be related to total exposure time (i.e., 3 in similar damage to 1 xylene exacerbated the dermatotoxic effects of xylene. Our data indicate that dermal penetration, but not the rate of dermal permeation, is directly proportional to exposure time; alterations in permeation rate are associated with the dermatotoxic effects of xylene; the stratum corneum and the epidermis are both barriers to xylene; xylene forms reservoirs in the skin even after short exposures; and the dermis is important as a reservoir only after permeation through the epidermis is no longer rate limiting.

Ertel KD, Neumann PB, Keswick BH, Kligman AM, Stoudemayer T. **A comparison of two antecubital fossa tests with personal care products**. J Toxicol Cutan Ocular Toxicol 1997;16(1):19-30.
BIOSIS COPYRIGHT: BIOL ABS. Two antecubital wash protocols were compared by testing a syndet bar and a moisturizer. The results show that the irritation potential predicted for a material is highly dependent on the conditions under which it is tested. It is possible to generate irritation far in excess of what would be expected under normal use, even for a product with skin-healing effects, by using aggressive exposure conditions. The results highlight a need to employ exposures representative of the anticipated use conditions and to interpret results in the context in which they are generated to avoid drawing incorrect conclusions about a material's in-use irritancy.

Eser HP, Potsch L, Skopp G, Moeller MR. **Influence of sample preparation on analytical results: drug analysis (GC/MS) on hair snippets versus hair powder using various extraction methods**. Forensic Sci Int 1997;84(1-3):271-9.
BIOSIS COPYRIGHT: BIOL ABS. The comparison of aqueous extraction methods and hair extraction by organic solvents performed on hair powder as well as on hair snippets of the same sample revealed different qualities of the procedures. Qualitative and quantitative results by the same derivatization step and GC/MS detection demonstrated, that the risk of missing a drug substance is higher using hair snippets than after drug extraction on pulverized hair. Drug recovery for opiates, cocaine and benzoylecgonine from hair was found to be best in aqueous solvents or in methanol extracts. The results are discussed under the aspects of solid-phase extraction, the hair sample representing an inhomogeneous material. The localization of drug molecules in hair, the hair swelling and penetration behavior of the particular extraction medium as well as the partition coefficient of solvent/hair phase for a particular drug substance.

Fang JY, Huang YB, Wu PC, Tsai YH. **Transdermal iontophoresis of sodium nonivamide acetate. Part 1. Consideration of electrical and chemical factors**. Int J Pharm 1996 Oct 25;143:47-58.
IPA COPYRIGHT: ASHP The influence of electrical and physicochemical factors on the kinetics of transdermal iontophoresis of sodium nonivamide acetate was investigated in vitro using shaved rat skin. Iontophoresis increased the transdermal penetration flux of nonivamide as compared to passive diffusion. Various modes for the application of electric currents possessing the same electrical energy were used. The iontophoretic flux of nonivamide decreased as donor solution ionic strength and donor buffer pH values increased. The discontinuous on/off cyclic application mode showed higher penetration capacity than the continuous mode.

Fang JY, Wu PC, Huang YB, Tsai YH. **In vitro permeation study of capsaicin and its synthetic derivatives from ointment bases using various skin types**. Int J Pharm 1995 Dec 29;126:119-28.
IPA COPYRIGHT: ASHP The in vitro penetration of nonivamide and sodium nonivamide acetate through rat skin from ointment bases and the relative skin permeability of capsaicin, nonivamide (NVA), and sodium nonivamide acetate (SNA) through different animal skin types were studied. Oil-in-water emulsion-type bases revealed better percutaneous absorption effects than others studied for both NVA and SNA. In comparison of in vitro permeability through various animal skin types, full-thickness human skin showed the poorest permeability for NVA, SNA, and capsaicin. The trends of steady state flux through the various skin types for the 3 compounds were quite different. However, pig skin could be successfully used as a model to study in vitro percutaneous absorption of these 3 compounds through human skin.

Fincher TK, Yoo SD, Player MR, Sowell JW, Michniak BB. **In vitro evaluation of a series of N-dodecanoyl-L-amino acid methyl esters as dermal penetration enhancers**. J Pharm Sci 1996 Sep;85:920-3.
IPA COPYRIGHT: ASHP The synthesis of 10 N-dodecanoyl-L-amino acid methyl esters and n-pentyl N-acetylprolinate was described and their in vitro penetration enhancement on a saturated suspension of hydrocortisone in propylene glycol, applied to excised hairless mouse skin, was investigated using a diffusion cell technique; enhancers were applied 1 h before drug treatment. Enhancement ratios (ER) were determined for the permeability coefficient (P), 24 h diffusion cell receptor concentration (Q24), and 24 h full thickness skin steroid content (SC). The highest values for P (ER 13.9) and Q24 (ER 13.7) were obtained with a proline ester. An alanine derivative showed the highest SC value (ER 16.5).

Franke P, Hoffmann K, Tauber U, Keipert S. [**Studies on the in vitro release of steroids from ointments**]. Pharm Ind 1996;58(12):1152-6. (Ger)
IPA COPYRIGHT: ASHP An in vitro model utilizing a Franz diffusion cell was developed and optimized for low liberation profiles to determine the release of methylprednisolone aceponate from Advantan fatty ointment, ointment, and cream; 4 synthetic membranes and various forms of impregnation and acceptor media were tested. A Cuprophan membrane, impregnated with the acceptor medium methyl alcohol (methanol)/phosphate buffer (1:1, pH 5) proved to be particularly suitable. The release profiles obtained for fatty ointment, ointment, and cream or solution corresponded in their ranking to the results recorded in in vitro experiments on full thickness skin of hairless mice. It was concluded that the method tested provides reproducible results with only slight variance and is suitable for assessing pharmaceutical quality in the development and optimization of corresponding cutaneous preparations.

Frankild S, Basketter DA, Andersen KE. **The value and limitations of rechallenge in the guinea pig maximization test**. Contact Dermatitis 1996;35(3):135-40.
The guinea pig maximization test (GPMT) has played a primary role in the evaluation of potential skin contact sensitizers for 25 years. In the OECD Guideline 406 from 1993, it is specifically suggested that equivocal results from the initial challenge in the GPMT should be evaluated further with a repeated challenge. However, there exist few published rechallenge data and the guideline does not describe how rechallenge data should be interpreted. In this paper, we have used examples from published results to illustrate both the positive value and the limitations of repeated challenges, including cross challenge. Testing with modified concentrations may also help to indicate whether or not the response is allergic in nature, particularly where there has been a low level of skin reaction observed in shamtreated controls, or where a low level of skin reaction is the dominant response in the test animals. In conclusion, the data presented demonstrate that, as a tool for the investigation of skin sensitizing potential, the GPMT can benefit from an experienced scientific evaluation of rechallenge data, but that this information should not be treated in a mechanistic fashion.

Fuhrman LC Jr, Michniak BB, Behl CR, Malick AW. **Effect of novel penetration enhancers on the transdermal delivery of hydrocortisone: an in vitro species comparison**. J Control Release 1997;45(2):199-206.
CBAC COPYRIGHT: CHEM ABS Six novel compds. were examd. for enhancer activity using occluded hairless mouse skin (HM), hairless rat skin (HR), human cadaver skin (HC) in vitro with hydrocortisone as the model drug. The compds. investigated included: N-dodecyl-2-pyrrolidinone (DPY), N-dodecyl-2-piperidinone, N-dodecyl-N-(2-methoxyethyl)acetamide, N-dodecyl-N-(2-methoxyethyl)isobutyramide, N-dodecyldiethanolamine, 2-(1-nonyl)-1,3-dioxolane, and Azone. Controls consisted of no enhancer or vehicle treatment. All enhancers were applied at 0.4M in propylene glycol 1 h prior to skin application of a satd. suspension of hydrocortisone in the same vehicle. Enhancement ratios (ER) were detd. for 24 h diffusion cell receptor concns. (Q24), permeability coeffs. (P), and 24 h full-thickness skin steroid contents. ER for controls was 1.0. DPY, an Azone analog, showed the greatest ER values for permeability coeff. (HM: 21.3, HR: 20.7, HC: 8.0) compared to control (ER: 1.0) and Azone (HM: 18.0, HR: 13.1, HC: 5.5) in all 3 animal skin models. All 6 enhancers exhibited poor skin steroid retention (compared to Azone) in all 3 skin models.

Fujii M, Yamanouchi S, Hori N, Iwanaga N, Kawaguchi N, Matsumoto M. **Evaluation of Yucatan micropig skin for use as an in vitro model for skin permeation study**. Biol Pharm Bull 1997;20(3):249-54.

Hansen E, Sclafani J, Liu P, Nightingale J. **The effect of water on a new binary transdermal flux enhancer (Peg3-Me/IPP): an in vitro evaluation using estradiol**. Drug Dev Ind Pharm 1997;23(1):9-14.
CBAC COPYRIGHT: CHEM ABS Iso-Pr palmitate (IPP), a skin penetration enhancer, combined with triethylene glycol monomethyl ether (Peg3-Me) results in an excellent transdermal flux enhancer. A soln. of 11% Peg3-Me/IPP satd. with estradiol delivered the drug at a 60-fold greater rate than from estradiol (E2)-satd. donors of IPP or Peg3-Me alone. Unfortunately, a steady-state flux is not maintained. Studies using vertical permeation cells indicated that the back flux of water causes the donor soln. to phase sep., with an IPP rich phase floating away from the skin. The ternary phase diagram for IPP, Peg3-Me, and H2O shows that a soln. of IPP/Peg3-Me will only accept 1% water before phase sepg. Addnl. expts., involving donor soln. replacement and reorientation of the skin relative to the donor soln., demonstrated that phase sepn. was responsible for the non-steady-state E2 flux. Finally, a prototype bilayer laminate, which included a hydrophobic polyisobutylene layer (PIB), minimized the water flux from the receiver chamber into the donor and produced a sustained and high transdermal flux. While the mechanism of enhancement is complex, the PEg3-Me/IPP flux enhancers may provide significant improvements for transdermal drug delivery.

Huang YB, Wu PC, Ko HM, Tsai YH. **Effect of pretreatment by cardamom oil on in vitro percutaneous penetration of piroxicam gel**. Int J Pharm 1996 Apr 19;131:137-41.
IPA COPYRIGHT: ASHP The effect of pretreatment by the penetration enhancer, cardamom oil, on the percutaneous penetration of piroxicam from gel through rabbit abdominal skin was investigated using an in vitro technique. The flux and the cumulative amount after 1 pretreatment with 10% cardamom oil in 3 vehicle systems (alcohols, alcohol/pH 5.8 buffer, and alcohol/pH 7.4 buffer) were higher than that of nonpretreatment, and were similar to that of 3 or 6 h pretreatment. A specific correlation between piroxicam flux and the pretreatment period was found. Compared to the lag time of skin penetration of piroxicam for nonpretreatment, the lag time for pretreatment was remarkably diminished. The penetration index of piroxicam after 1 h pretreatment with 10% cardamom oil in alcohol/pH 7.4 buffer was 340.9-fold higher than that of nonpretreatment. However, 1 h pretreatment with 10% cardamom oil in alcohol had no significant enhancing effect on piroxicam percutaneous penetration from gel. It was assumed that cardamom oil increased percutaneous penetration of piroxicam by direct effects on the barrier nature.

Kamimura W, Ooya T, Yui N. **Interaction of supramolecular assembly with hairless rat stratum corneum**. J Control Release 1997;44(2-3):295-9.
CBAC COPYRIGHT: CHEM ABS Polyrotaxanes are well known as a supramol. assembly in which many cyclic compds. are threaded onto a linear polymeric chain capped with bulky end-groups. In this paper, a polyrotaxane consisting of alpha-CDs and PEG capped with biodegradable peptide moieties was synthesized, and the interaction with stratum corneum of hairless rat skin was examd. by means of a differential scanning calorimetry. The hydroxypropylated polyrotaxane was found to interact with lipid components in the stratum corneum: bound water content was significantly decreased although ordered lipid bilayers were maintained. Thus, it is suggested that our designed polyrotaxane can be feasible as novel candidates for transdermal penetration enhancers.

Kim M, Chung S, Lee M, Cho A, Shim C. **Targeted and sustained delivery of hydrocortisone to normal and stratum corneum-removed skin without enhanced skin absorption using a liposome gel**. J Control Release 1997;46(3):243-52.
CBAC COPYRIGHT: CHEM ABS A liposome-gel formulation contg. 1% hydrocortisone was prepd. by blending phosphatidylcholine liposomes of hydrocortisone with Carbopol 934 hydrogel. The liposome-gel was applied topically onto the normal and stratum corneum (SC)-removed skins (3.0 cm2) of hairless mice at a dose of 1 mg as hydrocortisone. Percutaneous absorption of hydrocortisone across the SC-removed skin was significantly faster than that across normal skin, suggesting that SC behaves as a penetration barrier for the liposome-bound drugs.

Contrary to previous reports that have suggested enhanced percutaneous penetration of drugs by liposomes, the liposome-gel in this study reduced the skin absorption of hydrocortisone, compared with the conventional ointment formulation. The amt. of hydrocortisone absorbed from the liposome-gel after 8 h into the SC-removed skin was less than one-third of that from the conventional ointment. In spite of the reduced absorption, higher and sustained skin concns. of hydrocortisone were achieved for the liposome-gel as compared to the ointment. Drug concn. in both viable and deep skin reached its max. within 0.5 h after application of both formulations to both skin types. Drug concns. in both skins from the ointment declined as a function of time, while those from the liposome-gel were greatly sustained. The sustainment by the liposome-gel was more remarkable in the viable skin than in the deep skin. Drug concn. in the viable skin could be maintained at a nearly const. level for over 8 h by applying the liposome-gel. As a result, a 5-fold higher viable skin drug concn. was obtained from the liposome-gel than from the ointment at 8 h after the application to the SC-removed skin. Nevertheless, the plasma concn. of hydrocortisone at 4 h from the liposome-gel was only one-fourth the value from the ointment when the drug was applied to the SC-removed skin. Thus, retarded diffusion of the drug from the skin to the systemic blood stream appears to be a potential factor in the sustained skin concn. of hydrocortisone from the liposome-gel. The retarded diffusion was supported by the lower urinary (one-third) and fecal (one-half) excretion of the drug from the liposome-gel as compared to the ointment when the drug was applied to SC-removed skin. Interaction of hydrocortisone in the skin with phosphatidylcholine, a component of the liposomes and skin, may well be a factor in retarding the diffusion of the drug in the skin.

Kurnik RT, Potts RO. **Modeling of diffusion and crystal dissolution in controlled release systems**. J Control Release 1997;45(3):257-64.
CBAC COPYRIGHT: CHEM ABS New math. models have been derived for the controlled release of pharmaceutical compds. from a transdermal patch with simultaneous dissoln. of crystals. The math. model used for a 1-layer system is for a finite length slab with dissoln. An anal. soln. for this system, assuming const. crystal surface area was obtained. A numerical soln. for this system, allowing for a variable surface area of the crystal as it dissolves was also obtained. The models have also been extended to 2-layer systems, (patch + skin) where the first layer contains crystals of variable area (decreasing as the crystals dissolve). These models were solved by numerical methods using finite difference techniques. The models were applied to release of estradiol from a polymeric matrix into water and through skin. The estradiol consisted of sol. and crystal forms, with the crystals dispersed throughout the polymer. The crystal size has a significant impact of release of estradiol into water, with roughly a 40% increase in the amt. delivered as the crystal size was decreased from 3 to 0.5 .mu.m. When estradiol was delivered transdermally, however, it was found that there is essentially no effect of crystal size on the delivery of estradiol, due to the rate limiting nature of the skin membrane. Consequently, the most likely applications of dispersed phases in the drug delivery industry will be in the area of implanted or mucosal systems, as the stratum corneum will not be a barrier in these cases and the dispersed solid may serve as a drug reservoir for the sol. drug as it is depleted. When the system is used in this manner, a more compact design is possible than if all the drug had to be present initially as a sol. compd.

Lee AJ, King JR, Barrett DA. **Percutaneous absorption: a multiple pathway model**. J Control Release 1997;45 (2):141-51.
CBAC COPYRIGHT: CHEM ABS The aim of this study is to present a math. model of percutaneous absorption which considers the simultaneous penetration of drug by transcellular and intercellular pathways, as well as movement of drug between these two pathways. A 2-layer math. model is formulated and solns. to this model are obtained numerically for data sets contg. a range of physicochem. parameters relating to the percutaneous pathways and model drugs. Detailed graphical output is presented of the drug concn. profiles with skin depth, the effect of lipophilicity on steady state fluxes and the total amt. of drug delivered, and the formation of drug depots within the skin. Many qual. similarities exist between the multiple pathway model presented and previously proposed single and uncoupled pathway math. models. However, the model presented reveals effects of changes to the physicochem. properties of the penetrant or the skin structure which are not shown with a single or uncoupled pathway model.

Lewis RW, Basketter DA. **Transcutaneous electrical resistance: application in predicting skin corrosives**. Curr Probl Dermatol 1995;23:243-55 .

Lin RY, Ou YC, Chen WY. **Role of electro-osmotic flow on in vitro transdermal iontophoresis**. J Control Release 1997 Jan;43:23-33.
IPA COPYRIGHT: ASHP The role of electroosmotic flow in transdermal iontophoresis was studied in vitro with porcine skin using amino acids and their amino or carboxyl group blocked derivatives as model compounds. The electroosmotic flow direction enhanced the permeation of positively charged solutes, and enhancement was diminished as buffer salt concentration increased. For anionic and neutral solutes, the permeation reduced by increasing the buffer salt concentration was not as apparent as for cationic solutes. The increase in electrical current density raised the permeation of cationic solutes by increasing electrical and electroosmotic flow. For anionic solutes, the favor of electrical flow in a higher electrical field was weakened by increasing negative electroosmotic flow. The hydrophobicity of the solutes played a more prominent role than molecular weight in passive diffusion.

Loftsson T, Sigurdardottir AM. **Cyclodextrins as skin penetration enhancers. Effects of polymers on cyclodextrin complexation and transdermal drug delivery**. Proc Int Symp Cyclodextrins, 8th 1996;403-6.
CBAC COPYRIGHT: CHEM ABS The flux of hydrocortisone and enalaprilat was detd. from aq. vehicles contg. various amts. of 2-hydroxypropylbetaCD, carboxymethylbetaCD, randomly methylated betaCD or maltosylbetaCD through hairless mouse skin. When the drug was in suspension, the flux was increased as the cyclodextrin (CD) concn. was increased. The flux decreased at higher CD concns., when all the drug was in soln. Maximum flux through the skin was obtained when just enough CD was used to keep all the drug in soln. Addn. of small amt. of a water-sol. polymer, such as hydroxypropyl Me cellulose or polyvinylpyrrolidone, to the aq. complexation medium, and heating in sealed container to 120-140.degree.C for 20-40 min, resulted in up to 200% larger drug permeability compared to prepns. contg. no polymer.

Michel-Buono M, Buono JP, Serre G, Dumont D, Bernard P. **In vitro cytotoxic effects of 4,4'-bipyridyl on normal human keratinocytes**. Cell Biol Toxicol 1997;13(3):193-204.
BIOSIS COPYRIGHT: BIOL ABS. Recent epidemiological studies have brought to light a possible link between premalignant or neoplastic skin lesions (Bowen disease, squamous carcinoma) and occupational exposure to 4,4'bipyridyl (4,4'B), a precursor in the synthesis of paraquat herbicide. The present study used a serum-free cell culture of normal human keratinocytes (NHK) and two skin-equivalent models to test the effects of exposure to different concentrations of 4,4'B. Cytotoxicity of 4,4'B on NHK was measured by neutral red release assay. Superoxide dismutase (SOD) activity and cell cycle were analyzed in exposed and nonexposed NHK cultures. Histological and immunohistological tests enabled evaluation of differentiation and proliferation effects in reconstructed-skin models. Results showed that significant cytotoxicity occurred after 5 to 11 days' exposure to 4,4'B concentrations of 10-6-10-3 mol/L (IC50 between 10-3 and 10-4 mol/L 4,4'B after 11 days). Parallel modifications of SOD activity were recorded. Histological and immunohistological analysis revealed dose-related 4,4'B effects in reconstructed skin models. This involved abnormal terminal differentiation, connected with filaggrin expression, observed in skin models exposed to 10-7 and 10-6 mol/L 4,4'B. However, no modification of cell cycle or dysplasia was detected as a result of exposure to 4,4'B. Thus, 4,4'B appears to be cytotoxic for NHK, but as an isolated contaminant, and is unable to induce keratinocyte dysplasia in vitro. These preliminary results do not exclude a cocarcinogenic action of 4,4'B (with UVB for example).

Myers RC, Ballantyne B. **Comparative acute toxicity and primary irritancy of various classes of amines**. Toxic Subst Mech 1997;16(2):151-93.

Nakamura Y, Takayama K, Higashiyama K, Suzuki T, Nagai T. **Promoting effect of O-ethylmenthol on the percutaneous absorption of ketoprofen**. Int J Pharm 1996;145(1-2):29-36.
CBAC COPYRIGHT: CHEM ABS The promoting effect of O-ethylmenthol (MET) on the percutaneous absorption of

ketoprofen from alc. hydrogels was evaluated in rats in vitro and in vivo. Further, the anti-inflammatory action of ketoprofen hydrogels was evaluated with a rat paw edema test. The time course of the cumulative amts. of drug permeated through the rat skin in vitro exhibited a linear relation after an initial time lag. This was analyzed in a membrane diffusion model and the diffusion and partition parameters of ketoprofen were estd. Both parameters were remarkably enhanced when a hydrogel contg. a small quantity of MET (0.5%) was applied. However, at least 2% menthol was required to obtain the same activity as 0.25% MET. A pharmacokinetic model, which was derived on the assumption of a const. penetration rate (Rp) after a lag time, was employed to evaluate in vivo percutaneous absorption of ketoprofen from hydrogels contg. MET. Further, the area under the plasma concn.-time curve (AUC0-8h) was estd. In order to obtain the significant inhibitory action of ketoprofen on the rat paw edema induced by carrageenan, at least 1% menthol was required in the hydrogel formulation. On the other hand, a small amt. of MET (0.25-0.5%) was enough to bring about significant inhibitory action of ketoprofen. Distinguishable changes of the skin surface were microscopically obsd. with 0.5-2% MET, i.e. the spaces between the stratum corneum cells became extended and the shape of each cell became clear, whereas the morphol. changes caused by menthol were relatively weak. Both MET and menthol may change the dense barrier structure of the stratum corneum of skin; however, the efficiency of MET is significantly greater than that of menthol.

Neumann NJ, Hoelzle E, Lehmann P, Rosenbruch M, Klaucic A, Plewig G. **Photo hen's egg test: a model for phototoxicity**. Br J Dermatol 1997;136(3):326-30.
BIOSIS COPYRIGHT: BIOL ABS. The aim of this investigation was to establish a new model for phototoxicity which is more advanced than the widely used cultures of yeasts, bacteria or cells of various origin, and at the same time to avoid animal testing. We studied the extraembryonal vasculature of the incubated hen's egg. This model was originally introduced by toxicologists as an alternative to the rabbit's eye irritation test (Draize test). In the photo hen's egg test, substances are applied to the embryo's yolk-sac blood vessel system at a non-toxic concentration and are irradiated with 5 J/cm2 ultraviolet A (UVA) (320-400 nm). Promethazine, hematoporphyrin, ciprofloxacin and 8-methoxypsoralen were tested in this system. Death of the embryo, membrane discoloration and haemorrhage are parameters for phototoxic damage, which were recorded during an observation period of 24 h. These well-known phototoxic substances induced pronounced damage of the yolk-sac membrane and blood vessels which was not found in the controls (test substance alone, UVA alone or untreated) using a 2s egg test serves as a valid screening model for substances supposed to be photosensitizers owing to a phototoxic mechanism.

Nichols JW, Mckim JM, Lien GJ, Hoffman AD, Bertelsen SL, Elonen CM. **A physiologically based toxicokinetic model for dermal absorption of organic chemicals by fish**. Fundam Appl Toxicol 1996; 31(2):229-42.
A physiologically based toxicokinetic model was developed to describe dermal absorption of waterborne organic chemicals by fish. The skin was modeled as a discrete compartment into which compounds diffuse as a function of chemical permeability and the concentration gradient. The model includes a countercurrent description of chemical flux at fish gills and was used to simulate dermal-only exposures, during which the gills act as a route of elimination. The model was evaluated by exposing adult rainbow trout and channel catfish to hexachloroethane (HCE), pentachloroethane (PCE), and 1,1,2,2-tetrachloroethane (TCE). Skin permeability coefficients were obtained by fitting model simulations to measured arterial blood data. Permeability coefficients increased with the number of chlorine substituent groups, but not in the manner expected from a directly proportional relationship between dermal permeability and skin:water chemical partitioning. An evaluation of rate limitations on dermal flux in both trout and catfish suggested that chemical absorption was limited more by diffusion across the skin than by blood flow to the skin. Modeling results from a hypothetical combined dermal and branchial exposure indicate that dermal uptake could contribute from 1.6% (TCE) to 3.5% (HCE) of initial uptake in trout. Dermal uptake rates in catfish are even higher than those in trout and could contribute from 7.1% (TCE) to 8.3% (PCE) of initial uptake in a combined exposure.

Ogiso T, Iwaki M, Tanino T, Nishioka S, Higashi K, Kamo M. I**n vitro skin penetration and degradation of enkephalin, elcatonin and insulin**. Biol Pharm Bull 1997;20(1):54-60.

Paquet I, Chouinard N, Rouabhia M. **Cutaneous cell and extracellular matrix responses to ultraviolet-B irradiation**. J Cell Physiol 1996;166(2):296-304.
BIOSIS COPYRIGHT: BIOL ABS. The present study examined fibroblasts and keratinocytes in monolayers and cultured within dermal and skin substitutes and their use in assessing the effect of UVB irradiation on cutaneous cells and extracellular matrix organization. Dermal substitutes (DS) were produced by incorporating normal fibroblasts into a collagen lattice and skin substitutes (SS) were obtained by seeding normal keratinocytes onto the DS. Keratinocyte monolayers, fibroblast monolayers, DS, and SS were exposed once a day to a UVB source (10 mJ/cm2). The irradiation protocol was stopped when the keratinocytes of the non-irradiated cultures (control groups) had reached confluence. Microscopic observations revealed that UVB radiation decreased both fibroblast and keratinocyte growth and enhanced their differentiation resulting in (1) less fibroblasts in the DS and SS, and (2) incomplete coverage of the DS by keratinocytes. Microscopic observations and histological analyses revealed major morphological changes. Both cell types became bigger and presented wide nuclei and vacuoles in the cytoplasm. No organized deep epidermal layer was observed in irradiated compared to non-irradiated SS. Irradiated DS and SS extracellular matrices showed an irregular aggregating collagen fiber organization with serious discrepancies suggesting large defects in the structural properties of the extracellular matrix. The present study demonstrated that exposure to a UVB source led to profound morphological and functional disturbances in both cutaneous cells and in the extracellular matrices of the DS and SS. The present technology would be of great interest for step-by-step studies of UVR effects on cutaneous cell morphology and functional properties, and could be an alternative to using animals for pharmacological and toxicological evaluations.

Patil S, Singh P, Szolar-Platzer C, Maibach H. **Epidermal enzymes as penetration enhancers in transdermal drug delivery**? J Pharm Sci 1996 Mar;85:249-52.
IPA COPYRIGHT: ASHP The feasibility of using epidermal enzymes to enhance the penetration of drugs across skin was evaluated in vitro using benzoic acid, mannitol, and testosterone as model drugs. The enzymes facilitated transdermal drug delivery. The effects were more pronounced for mannitol compared to benzoic acid and relatively lipophilic testosterone. Phospholipase C was the most effective of the enzymes studied, as it facilitated the transport of all solutes and to the greatest extent. Although not proven, the effects of the topical enzymes appeared to be mediated through their effects on skin lipids.

Pellett MA, Castellano S, Hadgraft J, Davis AF. **The penetration of supersaturated solutions of piroxicam across silicone membranes and human skin in vitro**. J Control Release 1997;46(3):205-14.
CBAC COPYRIGHT: CHEM ABS Under ideal circumstances, max. flux is achieved from satd. solns. and, therefore, the diffusion of compds. from supersatd. solns. would provide enhanced penetration. However, due to the inherent lack of stability of supersatd. solns., they tend to crystallize upon prepn., but in some cases this can be overcome with antinucleant polymers which inhibit or retard crystn. Supersatd. solns. of piroxicam for a range of different degrees of satn. up to 5.3 were prepd. in a 40:60, vol./vol., propylene glycol/water cosolvent mixt. Solns. up to 4 degrees of satn. were stable for at least 16 h and their penetration across silicone membranes and full-thickness human skin was investigated using diffusion cells. Relationships between flux and degree of satn. for the supersatd. systems produced linear correlations, but flux values across skin at 0.5 and 1 degrees of satn. were similar. This was partly attributed to differences in the soly. of the drug at different donor phase temps. Mechanisms of action for antinucleant polymers were considered, and it was postulated that hydroxypropylmethyl cellulose prevented the formation of a hydrate form of piroxicam, which was less sol. than its anhyd. form in aq. based solvent systems.

Perkins MA, Osborne R, Johnson GR. **Development of an in vitro method for skin corrosion testing**. Fundam Appl Toxicol 1996;31(1):9-18.
The feasibility of an in-vitro skin corrosion test method using commercially available human skin cultures was investigated. Skin2 from Advanced Tissue Sciences and EpiDerm from MatTek were evaluated for their responses to 24 corrosive and noncorrosive materials. The multilayered epidermis of the Skin2 cultures contained a basal layer of keratinocytes, a stratum corneum and a stromal component. The EpiDerm cultures did not have a dermal element, but had a stratified and cornified epithelium. Epithelial degradation and necrosis were graded on a scale of 0 to 5 after exposure to the test materials. Plastic embedding as opposed to paraffin embedding of Skin2 cultures

gave results comparable to those of EpiDerm cultures. Treatment of both cultures with corrosive materials produced significantly severe histological changes in under 3 minutes (min). Cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium-bromide (MTT) vital dye metabolism and found to be consistent for both skin cultures. A 50% reduction in cell viability, designated the t50 value, after less than a 3min exposure to the test substances was used to classify materials as corrosives. This method was used to evaluate nine corrosive chemicals and 15 skin irritants. The MTT assay results correlated with the histological grading of corrosive effects. The authors conclude that use of human skin cultures in corrosive evaluation provides a reliable replacement for the use of in-vivo animal testing.

Pirot F, Kalia YN, Stinchcomb AL, Keating G, Bunge A, Guy RH. **Characterization of the permeability barrier of human skin in vivo**. Proc Natl Acad Sci USA 1997;94(4):1562-7.
Attenuated-total-reflectance Fourier-transform-infrared spectroscopy has been used to rapidly and noninvasively quantify in vivo the uptake of a chemical into the outermost, and least permeable, layer of human skin (the stratum corneum). The objective of the experiment was to develop a general model to predict the rate and extent of chemical absorption for diverse exposure scenarios from simple, and safe, short-duration studies. Measurement of the concentration profile of the chemical in the stratum corneum, and analysis of the data using the unsteady-state diffusion equation, enabled estimation of the permeability coefficient and calculation of the time required to achieve maximal transdermal flux. Validation of the spectroscopic technique employed was established, and quantitation of chemical uptake into the stratum corneum was confirmed independently using trace amounts of radiolabeled chemical in conjunction with liquid scintillation counting and accelerator mass spectrometry. The results presented have pharmacological and toxicological implications, as the technology lends itself both to the prediction of transdermal drug delivery, and the feasibility of this route of administration, and to the assessment of risk after dermal contact with toxic chemicals.

Pugh WJ, Roberts MS, Hadgraft J. **Epidermal permeability--penetrant structure relationships. Part 3. Effect of hydrogen bonding interactions and molecular size on diffusion across the stratum corneum**. Int J Pharm 1996;138(Jul 26):149-65.
IPA COPYRIGHT: ASHP An analysis of the effect of hydrogen bonding interactions and molecular size on diffusion across the stratum corneum for compounds with zero or 1 hydrogen bonding groups and polyfunctional compounds is presented using various previously reported data for the compounds. Hydrogen bonding between permeant and the stratum corneum is a major negative determinant of the diffusion coefficient (D) across pathlength (h) of the stratum corneum (D/h). For the solvatochromic parameters alpha and beta, the stratum corneum is predominantly a hydrogen bond donor (alpha), rather than acceptor (beta), with alpha:beta equal to 0.6:0.4. Retardation coefficients (RCs) of the compound chemical groups are the characteristic hydrogen bonding potentials to the stratum corneum that slow diffusion and are calculated differently when alpha and beta are known and when they are unavailable. The maximal diffusion attainable by small, non-bonding molecules is about 0.03 cm/h. The rate declines rapidly as hydrogen bonding groups are introduced and reaches a very low minimum after about 4 groups. The D/h vs RC plot resembles an adsorption isotherm, and the data can be fitted using Langmuir's equation. The effect of molecular weight on diffusion is lower than previously reported.

Qiao GL, Brooks JD, Baynes RE, Monteiro-Riviere NA, Williams PL, Riviere JE. **The use of mechanistically defined chemical mixtures (MDCM) to assess component effects on the percutaneous absorption and cutaneous disposition of topically exposed chemicals. I. Studies with parathion mixtures in isolated perfused porcine skin**. Toxicol Appl Pharmacol 1996;141(2):473-86.
BIOSIS COPYRIGHT: BIOL ABS. Recently, attention has been directed to the risk assessment of cutaneous exposure to chemical mixtures rather than to only a single compound since this is the exposure scenario in the environment, residence, and work place. Using acetone or dimethylsulfoxide (DMSO) (80% in water) as a vehicle, percutaneous absorption and cutaneous disposition of parathion (PA) were studied following PA (40 mug/cm2) dosing on isolated perfused porcine skin as mechanistically defined chemical mixtures (MDCM) consisting of the surfactant sodium lauryl sulfate (SLS), the rubefacient methyl nicotinate (MNA), and the reducing agent stannous chloride (SnCl2). A full 2orial design was used to asses treatment effects and potential interactions. More radiolabel

was absorbed with DMSO than with acetone albeit an earlier peak flux time but lower peak flux was observed with acetone than with DMSO. The absorption flux rate profiles with DMSO continued increasing but bipeak-featured profiles were observed with acetone. SLS enhanced PA absorption with both DMSO and acetone. The presence of MNA in both vehicles blunted the absorption rate curves without significantly changing total absorption. SnCl2 blocked PA absorption and increased residue level on the skin surface and in the stratum corneum (SC). The venous flux profiles were mixture-dependent and highly reproducible within treatment groups. Higher level interactions were also noted. This study indicated multiple levels of interactive effects on PA absorption which must be incorporated into any effort to identify critical mechanisms which affect risk assessment of topically exposed mixtures. It was suggested that the chemicals selected in a topically applied mixture may have significant effects on the penetration/distribution pattern and percutaneous absorption profile of a toxicant/drug in the mixture. The MDCM approach may be useful in a screening or triage approach to identify mixture components which affect marker chemical absorption as well as identify potential mechanisms which deserve further attention. Risk assessment efforts could then be focused on those mixtures, containing these critical components, which would be expected to have the greatest penetration and absorption.

Rasmussen ES. [**Use of reconstructed human tissue models in toxicological and pharmacological investigations**]. Dan Veterinaertidsskr 1996;79(21):943-8. (Dan)
BIOSIS COPYRIGHT: BIOL ABS. Skin reconstructed in vitro was first used for treatment of large burns. Pure epidermal cell culture sheets or composite tissues made of epidermal cells cultured on dermal substitutes have been employed. Human keratinocytes grown at the air-liquid interface can differentiate to a multilayered epidermis with a thin stratum corneum. A dermal tissue equivalent can be constructed by growing fibroblast on a nylon net where the cells built up their own tissue matrix. Reconstructed skin models exhibits in vivo like morphological and functional characteristics, and they are used to study the development and physiology of the skin, wound healing processes, and the response of the skin or other organs to chemical or environmental stress. The models are metabolically active, and reproduce to a certain extent the barrier function of the skin. Co-cultures of keratinocytes and other cells, e.g. melanocytes, can be used for advanced studies of cell-to-cell interactions. Test substances can be applied topically to the tissue models, and their irritant potential can be evaluated by measuring changes in cellular metabolism or the release of proinflammatory mediators. Good in vitro/in vivo correlations have been obtained testing chemicals and finished products for corrosivity, ocular and dermal irritancy, and phototoxicity. Tissue models are used by industry for screening of new substances, and they appear to show promise as adjuncts or alternatives to several acute toxicological guideline tests.

Rohatagi S, Barrett JS, Mcdonald LJ, Morris EM, Darnow J, Disanto AR. **Selegiline percutaneous absorption in various species and metabolism by human skin**. Pharm Res 1997;14(1):50-5.
PURPOSE: A Selegiline Transdermal System (STS) is under development for indications which may not be optimally or safely treated with oral selegiline. Studies were conducted to evaluate the in vitro penetration and skin metabolism of selegiline in order to better understand the toxicological findings and the observed plasma levels of selegiline and its metabolites in animals and man. METHODS: In vitro penetration studies were conducted in four different species (male hairless mice, male and female rats, female dog and male Micropig) and compared to human skin. In another study, viable human skin was used to estimate the extent of metabolism of selegiline by human skin using Franz diffusion cells. RESULTS: Results indicated that female dog and male Micropig skin were reasonable animal models for 24 hour in vitro selegiline penetration through human skin. Penetration of selegiline through rat skin and hairless mouse skin was 2-fold and 3-fold higher than through human skin, respectively. Metabolism was negligible in human skin. Selegiline metabolites (L-methamphetamine and N-desmethylselegiline but not L-amphetamine) were detected at all time points but the extent of selegiline metabolism was negligible. The cumulative 24 hour in vitro selegiline permeation from a 1.83 mg/cm2 STS through human skin was 5.0 mg. This was similar to the in vivo permeation in humans as assessed by residual patch analysis. CONCLUSIONS: The similarity of dog and human skin permeation results support the use of the dog as a species for evaluating the toxicology of transdermally-administered selegiline. Selegiline is not metabolized cutaneously and hence the metabolic profile following STS administration is likely due to hepatic metabolism only.

Saied A, Makki S, Muret P, Humbert P, Millet J. **Psoralens percutaneous permeation across the human whole skin and the epidermis in respect to their polarity (in vitro study)**. J Dermatol Sci 1997;14(2):136-44.

Sato K, Mine T. **Analysis of in vitro rat skin permeation and metabolism of SM-10902, prodrug of synthetic prostacyclin analog**. Int J Pharm 1996 Jun 17;135:127-36.
IPA COPYRIGHT: ASHP The permeation and metabolism of SM-10902 (pimilprost), a prodrug of a synthetic prostacyclin analog (SM-10906), in rat skin were studied using a flow through type diffusion cell and were compared with those of SM-10906. Absorbed SM-10902 was entirely metabolized to the bioactive form, SM-10906, in the rat skin. The appearance of SM-10906 to the receptor was faster when applied as SM-10902 than when applied as SM-10906 in both types of intact and stripped skin. From the analysis of these permeation profiles by the 2-layer skin model with a metabolic pathway, the diffusion constants of SM-10902 in the stratum corneum and the lower layer were 70 and 6 times of those of SM-10906, respectively. However, the partition coefficient of SM-10902 from the ointment to the stratum corneum and the lower layer were equal to and twice as high as those of SM-10906, respectively. The appearance of metabolite to the receptor increased with the metabolic rate and reached a maximum point and thereafter decreased to a plateau level. According to this simulation, it was shown that SM-10902 has a favorable characteristic in terms of metabolic rate.

Schneider I, Dobner B, Neubert R, Wohlrab W. **Evaluation of drug penetration into human skin ex vivo using branched fatty acids and propylene glycol**. Int J Pharm 1996;145(1,2):187-96.

Singh P, Roberts MS. **Local deep tissue penetration of compounds after dermal application: structure-tissue penetration relationships**. J Pharmacol Exp Ther 1996;279(2):908-17.

Spielmann H, Liebsch M, Pape WJ, Balls M, Dupuis J, Klecak G, Lovell WW, Maurer T, De Silva O, Steiling W. **EEC/COLIPA in vitro photoirritancy program: results of the first stage of validation**. Curr Probl Dermatol 1995;23:256-64.

Sun GQ, Ping QN, Li C. [**Effect of penetration enhancers on the in vitro permeability of artesunate through excised mouse skin**]. J China Pharm Univ 1996;27(6):345-9. (Chi)
IPA COPYRIGHT: ASHP The effect of 9 absorption enhancers and combinations were evaluated for effects on in vitro permeability of artesunate through excised mouse skin. The drug penetration process was described by zero-order kinetics.

Tanojo H, Bouwstra JA, Junginger HE, Bodde HE. **In vitro human skin barrier modulation by fatty acids: skin permeation and thermal analysis studies**.Pharm Res 1997;14(1):42-49.
AB - CBAC COPYRIGHT: CHEM ABS This study aims to elucidate the skin permeation enhancement and the skin perturbation effects of a no. of fatty acids, i.e. straight-chain satd. (SFA), monounsatd. (MUFA) and polyunsatd. acids (PUFA). The skin permeation enhancement effects were studied using human stratum corneum (SC) and p-aminobenzoic acid (PABA) as a model permeant. The fatty acids in propylene glycol (FA/PG) were applied according to a pre-treatment/co-treatment protocol. The perturbation effects were studied using DTA (DTA) on SC after pretreatment with FA/PG. SFA with 6 to 12 carbons exhibit a parabolic correlation between enhancement effect and chain-length, with a max. at nonanoic-decanoic acids (with 9 and 10 carbons). Nonanoic and decanoic acids exert barely noticeable effects on the thermal behavior of SC, suggesting that they easily mix with the skin lipids. All cis-6, 9-, 11- or 13-octadecenoic acids (MUFA) enhance the permeation of PABA to the same extent. DTA revealed that the cis-9- and 13-isomers form a sep. domain contg. mostly the pure fatty acids within the SC lipids and suppress the lipid transitions at 70.degree./80.degree.C. PUFA-linoleic (LA), alpha-linolenic (ALA) and arachidonic acids-enhance PABA permeation stronger than MUFA but addnl. double bonds do not further increase the degree of enhancement. LA and ALA form sep. domains but do not completely suppress the SC lipid transitions at 70.degree./80.degree.C. Increase in the enthalpy changes of 70.degree./80.degree. transitions linearly correlates

to the decrease in the permeability coeffs., suggesting that an increased perturbation of the skin lipids not necessarily has to yield an increased PABA permeation. The enhancement effects of fatty acids on the PABA penetration through SC are structure-dependent, assocd. with the existence of a balance between the permeability of pure fatty acids across SC and the interaction of the acids to skin lipids.

Tsai JC, Guy RH, Thornfeldt CR, Gao WN, Elias PM, et al . **Metabolic approaches to enhance transdermal drug delivery. Part 1. Effect of lipid synthesis inhibitors**. J Pharm Sci 1996;85(6):643-8.
IPA COPYRIGHT: ASHP The potential of certain inhibitors of lipid synthesis to enhance the transdermal delivery of lidocaine or caffeine as a result of their capacity to perturb barrier homeostasis in hairless mice was investigated. After acetone disruption of the barrier, the extent of lidocaine delivery and the degree of altered barrier function paralleled each other. Moreover, the further alteration in barrier function produced by either the fatty acid synthesis inhibitor RMI-14514 (5-tetradecyloxy-2-furancarboxylic acid), the cholesterol synthesis inhibitor fluvastatin, or cholesterol sulfate (cholesteryl sulfate) resulted in a further increase in lidocaine absorption. Coapplications of RMI-14514 and cholesterol sulfate together caused an additive increase in lidocaine uptake. Coapplications of RMI-14514 and fluvastatin together again delayed barrier recovery and increased drug delivery by about 8-fold vs delivery from a standard enhancing vehicle. It was concluded that modulations of epidermal lipid biosynthesis, following application of conventional, chemical penetration enhancers, cause a further boost in transdermal drug delivery.

Uchino T, Tokunaga H, Ando M. [**Effect of hematoporphyrin - UVA sensitization on content of hydroperoxides in three-dimensional cultured cells (Skin2)**]. Nippon Koshohin Kagakkaishi 1996;20(4):259-64. (Jpn)
CBAC COPYRIGHT: CHEM ABS Hematoporphyrin UVA hydroperoxide skin;Hydroperoxides Hematoporphyrin-UVA sensitization effect on hydroperoxides in skin culture cells;Photodynamic action PUVA; hematoporphyrin-UVA sensitization effect on hydroperoxides in skin culture cells;Photosensitizers PUVA; hematoporphyrin-UVA sensitization effect on hydroperoxides in skin culture cells Biological;Skin Hematoporphyrin-UVA sensitization effect on hydroperoxides in skin culture cells;UV A radiation Lhematoporphyrin-UVA sensitization effect on hydroperoxides in skin culture cells.

Williams PL, Thompson D, Qiao G, Monteiro-Riviere N, Riviere JE. **The use of mechanistically defined chemical mixtures (MDCM) to assess mixture component effects on the percutaneous absorption and cutaneous disposition of topically exposed chemicals. II. Development of a general dermatopharmacokinetic model for use in risk assessment**. Toxicol Appl Pharmacol 1996;141(2):487-96.
BIOSIS COPYRIGHT: BIOL ABS. We present a conceptual approach to a general comprehensive mathematical model to quantify percutaneous absorption of topically applied chemicals in complex mixtures on the basis of biophysical parameters estimated or measured using in vitro and ex vivo perfused skin preparations. This model addresses mechanistically defined chemical mixtures (MDCM) which consist of components selected because of their potential to modulate by various mechanisms the absorption of a marker toxic penetrant. This model accounts for observed toxicodynamic general and specific effects of chemicals, acting single or in concert, on the absorption of any or all components in a defined mixture. We have also included experimental data from an isolated perfused porcine skin flap study with topically applied parathion as the marker penetrant and acetone or DMSO as solvent, with methyl nicotinate as a potential rubefacient, sodium laurel sulfate as a surfactant, and stannous chloride as a reducing agent in order to provide an illustration of the application and performance of the model. This model supports the MDCM concept that defining and then simulating those components of a complex mixture that could have a significant impact on the absorption of a marker toxic compound would be a useful screening approach in the risk assessment of topical chemical mixtures. It may also be used to identify critical pathways where chemical mixture component interactions significantly modify the absorption of the penetrant of interest.

Wu PC, Huang YB, Lin HH, Tsai YH. **In vitro percutaneous absorption of captopril through excised rabbit skin**. Int J Pharm 1996 Oct 25;143:119-23.
IPA COPYRIGHT: ASHP The permeation characteristics of captopril from aqueous solution at various pH values of

McIlvaine buffer solutions and the permeation rates of a 1% solution of captopril, with or without 5% of various anionic, cationic, and nonionic surfactants, through excised rabbit skin were investigated in vitro. The surfactants included sodium lauryl sulfate (anionic), benzalkonium chloride and cetylpyridinium chloride (cationic), and polysorbate 20 (polyoxyethylene sorbitan monolaurate; Tween 20), polysorbate 40 (polyoxyethylene sorbitan monopalmitate; Tween 40), polysorbate 60 (polyoxyethylene sorbitan monostearate; Tween 60), and polysorbate 80 (polyoxyethylene sorbitan monooleate; Tween 80) (nonionic). The results indicated that the pH dependent skin permeability of captopril, a zwitterionic drug, may reflect the permselective property of the skin, dependent on the lipophilicity and/or diffusivity of the ionic species. All of the surfactants significantly increased the penetration enhancement of captopril. Sodium lauryl sulfate was the most effective, increasing the penetration rate about 58-fold.

Xue YY, Zhao J, Weng GY, Liu Y, Su M. [**Topical penetration of tretinoin in vitro from liposomal gel and its stability**]. J China Pharm Univ 1996;27(7):405-7. (Chi)
IPA COPYRIGHT: ASHP The transdermal penetration of tretinoin in liposomal and conventional gels was evaluated using excised mouse skin and the stability of the liposomal gel was also investigated. The liposome formulation improved the local effect of tretinoin compared with the conventional gel.

Yokomizo Y. **Effect of phosphatidylcholine on the percutaneous penetration of drugs through the dorsal skin of guinea pigs in vitro, and analysis of the molecular mechanism, using attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy.** J Control Release 1996;42(12):249-62.
IPA COPYRIGHT: ASHP The percutaneous penetration of a water-insoluble drug, prednisolone, and a water-soluble drug, diclofenac sodium enhanced by soybean phosphatidylcholine, was characterized using in vitro skin percutaneous penetration methods with excised guinea pig dorsal skin; attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy was used to analyze the molecular mechanism of enhancement of drug penetration. The C-H bond stretching absorbance frequency shifts in the stratum corneum (SC) induced higher and broader absorbance shifts, and a shift was dependent on soybean phosphatidylcholine concentrations. Percutaneous penetration of prednisolone was dependent on phosphatidylcholine concentration; however, percutaneous penetration of diclofenac was not dependent on phosphatidylcholine concentration. Prednisolone percutaneous penetration was proportional to C-H bond stretching absorbance frequency shifts, while percutaneous penetration of diclofenac was not. Prednisolone and diclofenac skin accumulation was inversely proportional to C-H bond stretching absorbance frequency shifts. It was concluded that transdermal water-insoluble drug penetration may be related to SC lipid structure.

ECOTOXICITY

Carr RS, Chapman DC, Howard CL, Biedenbach JM. **Sediment quality triad assessment survey of the Galveston Bay, Texas system**. Ecotoxicology 1996;5(6):341-64.
BIOSIS COPYRIGHT: BIOL ABS. To characterize the quality of sediments at key sites in the Galveston Bay Estuary, sediment samples were collected concurrently for chemical and physical analyses, toxicity testing and an assessment of benthic community structure. Significant toxicity, as determined by the sea urchin (Arbacia punctulata) pore water embryological development assay, was observed at 12 of the 24 sites investigated in this study. No toxicity was observed at any of the sites with the amphipod (Grandidierella japonica) solid-phase test. There were a number of sites with elevated levels of trace metals and petroleum hydrocarbons. The chemistry, toxicity and benthic data were ranked by station and a scaled rank sum was calculated to facilitate comparisons among the stations. Five sites exhibited strong evidence of contaminant-induced degradation, while 15 stations showed no evidence of contaminant-induced degradation. At eight additional sites the sediment quality triad (SQT) data indicated that unmeasured chemicals or conditions were stressing the system. Contaminant impacts could be reduced or eliminated by alternative regulatory and management practices, including the restriction of produced

water discharges into coastal estuaries and the use of dredge material disposal practices that minimize the reintroduction of sediment-associated contaminants to the bays.

Cheung YH, Neller A, Chu KH, Tam NF, Wong CK, Wong YS, Wong MH. **Assessment of sediment toxicity using different trophic organisms**. Arch Environ Contam Toxicol 1997;32(3):260-7.
The main aim of the present project is to study the feasibility of using different trophic organisms for evaluating the toxicity of dredged sediments arising in Hong Kong. A total of eight sediment samples (duplicate samples collected from four selected sites: Kowloon Bay, Tsing Yi,Chek Lap Kok, and Double Haven) of Hong Kong coastal waters were analyzed for the total concentrations of As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn, total organic carbon, acid volatile sulfides, simultaneously extracted metals, redox potential, and 12 organic micropollutants. The sediment elutriates were also analysed for the various metal concentrations, as well as contents of ammonia-N, nitrate, total sulfide, sulfate, and total organic carbon. Elutriate Sediment Toxicity Tests (ESTT) were also conducted, using two microalgae (Skeletonema costatum, a diatom and Dunaliella tertiolecta, a flagellate), juvenile shrimp (Metapenaeus ensis) and juvenile fish (Trachinotus obtaus). Two commercially available tests using bacteria (Microtox Test and Toxi-Chromotest) also were employed to test both the solid phase and elutriates of the sediments. The results of Microtox test on the solid phase, and bioassay tests using diatom on the sediment elutriate, especially the former, were correlated significantly ($p < 0.05$)with a number of physico-chemical properties of sediments and elutriates. It is recommended that a combination of a liquid-phase bioassay using diatom and a solid-phase bioassay using microtox test should be used for screening a large number of sediment samples. However, the presence of ammonia in the sediments containing a high content of organic matter seemed to interfere the detection of contamination impacts.

Dijkman NA, Van Vlaardingen PL, Admiraal WA. **Biological variation in sensitivity to N-heterocyclic PAHs: effects of acridine on seven species of micro-algae**. Environ Pollut 1997;95(1):121-6.
CBAC COPYRIGHT: CHEM ABS The toxicity of the N heterocyclic polyarom. hydrocarbon (PAH) acridine was tested for seven species of microalgae: Scenedesmus acuminatus, Selenastrum capricornutum, Chlamydomonas eugametos, Staurastrum chaetoceras, Staurastrum manfeldtii, Navicula salinarum and Nitzschia sigma. The effect of acridine on the algae was studied in a 96-h growth test, in which growth rates were detd. using cell nos. and biovolume. The obtained EC50 values (for growth rates based on cell nos.) ranged from 0.08 mg litre-1 for N. sigma to 0.78 mg litre-1 for C. eugametos and N. salinarum. Effect concns. based on biovolume were slightly higher for most species. Metab. of acridine was obsd. for one species (S. capricornutum), but this capacity did not result in a very different tolerance. Acridine toxicity was neither related to taxonomical background (green algae vs. diatoms) nor to original habitat of the species (planktonic or benthic, eutrophic or oligo-meso-trophic). The presence of near-UV radiation during the incubation might explain the higher toxicity of acridine than is expected on basis of QSAR derived narcotic toxicity.

Donkin P, Widdows J, Evans SV, Staff FJ, Yan T. **Effect of neurotoxic pesticides on the feeding rate of marine mussels (Mytilus edulis)**. Pestic Sci 1997;49(2):196-209.
BIOSIS COPYRIGHT: BIOL ABS. The effects of selected neurotoxic pesticides on the feeding rate of marine mussels (Mytilus edulis) were determined. Two organochlorine pesticides, lindane and endrin, two acetylcholinesterase-inhibiting compounds, dichlorvos and carbaryl and two pyrethroids, flucythrinate and permethrin, were studied. No evidence was found for any specific neurotoxic effect of the organochlorines and pyrethroids on feeding efficiency. In contrast, dichlorvos and carbaryl inhibited the enzyme acetylcholinesterase in mussel gills and were more toxic to feeding efficiency than could be explained by a narcotic mechanism of toxicity alone. Dichlorvos also caused clear behavioural changes in the mussels. The significance of these observations for the application of mussels to impact assessment in the marine environment is discussed.

El Jarjaf A, Garnier J. [**Toxicological action of nitrates and heavy metals (Cd2+ and Cu2+) on two freshwater algae**]. J Eur Hydrol 1996;27(2):213-25. (Fre)

Ferguson LR, Gregory TJ, Pearson AE, Hay JE, Lewis GD. **Mutagenicity tests as a monitoring tool for potential mutagens and carcinogens in shellfish gathering areas of New Zealand**. N Z J Marine Freshwater Res 1996;30 (4):413-21.
BIOSIS COPYRIGHT: BIOL ABS. Shellfish may bioaccumulate a variety of chemicals, some of which are mutagenic or carcinogenic to humans. Mutagenicity tests provide an integrated way of detecting these chemicals. This paper describes the application of two such tests to New Zealand shellfish in laboratory and field situations. The bacterial mutagenicity test gave positive results on a nitric acid extract of green-lipped mussels (Perna canaliculus) that had been exposed to model carcinogens under laboratory conditions. When applied to Pacific oysters (Crassostrea gigas) that had been sampled from four different sites in the Manukau Harbour, the same methods detected mutagenic activity which varied both by date and area sampled. The micronucleus assay gave a readily scored measure of chromosome damage in gill tissues in both mussels and oysters, but presented some practical problems in field studies. Our studies emphasise the need to sample within a short time interval, and the advantage of using a complementary package of bacterial mutagenicity and gill micronucleus assays.

Hartwell SI. **Demonstration of a toxicological risk ranking method to correlate measures of ambient toxicity and fish community diversity**. Environ Toxicol Chem 1997;16(2):361-71.
BIOSIS COPYRIGHT: BIOL ABS. The goal of this study was to assess a new toxicological risk ranking model, field validate it with results from a battery of sediment and water column bioassays, and identify correlations of model output with fish community and population metrics. The model has five components: severity of effect, degree of response, bioassay variability, consistency, and number of measured endpoints. The model can reliably reduce an array of ambient toxicity data into a site-specific metric that is appropriate for comparisons with other metrics, such as Index of Biotic Integrity (IBI) or community diversity indices. The model is tolerant of variable amounts of data between stations. It does not generate probability limits without repeated sampling. The model can identify trends between sampling stations and document where of cell lines is rapid and consistent conditions for culture are easily maintained; most of the phenotypic variation that is encountered with use of cell donors is eliminated; and radiolabeled precursors can be used for labeling and quantifying protein and DNA. We describe the current state of development of in vitro toxicity testing methods, present detailed procedures for the test methods optimized in our lab., and compare these techniques with other approaches. Toxicity testing using cell lines provides a mechanism to quantify the risks assocd. with environmental exposure to chem. mixts.

Hattori T, Shizuri Y. **A screening method for antifouling substances using spores of the fouling macroalga Ulva conglobata Kjellman**. Fish Sci 1996;62(6):955-8.
CBAC COPYRIGHT: CHEM ABS Fouling macroalgae cause serious problems by settling on ships' hulls, power plant cooling systems, aquaculture systems, and other marine infrastructure. In order to solve this problem, it is necessary to find antibouling substances without toxicity or with lower toxicity than that of toxic agents such as TBTO. For this purpose, an assay for antifouling substances is important. First, the effects of environmental factors on attachment and germination of spores of the fouling macroalga U. conglobata were examd. The optimum conditions for attachment and germination of spores were: temp., 20-25.degree.; salinity, 33; light intensity, 3000 lx under long-day regimen. Under such conditions, a new and efficient assay method for antifouling substances against the attachment of fouling macroalgae was developed. Antifouling activity was statistically examd.

Isomaa B, Lilius H. **The urgent need for in vitro tests in ecotoxicology**. Toxicol In Vitro 1995;9(6):821-5.
BIOSIS COPYRIGHT: BIOL ABS. Ecotoxicological risk assessment should be regarded as consisting of several elements including molecular biology, cell toxicology, toxicology, ecology, chemistry and computer modelling (including quantitative structure-activity relationship (QSAR) considerations). A successful risk assessment requires an approach that integrates data and knowledge from all the separate elements. The relative importance of a certain element will vary, depending on the situation or the chemical in question. One important, perhaps the most important, piece of information needed in risk assessment is the information about the concentration range at which a chemical exerts adverse effects on the organisms living in the aquatic environment. Without such information no prediction can be made or safety factors established. A combination of good in vitro tests could provide that information. A proper choice of endpoints in in vitro testing could also provide information on sublethal effects and

toxic mechanisms involved, enabling even a more efficient approach than whole animal testing. For aquatic in vitro toxicology to progress further, there is a need for a more integrated, a more comparative and a more mechanistic approach and for large-scale evaluations. It would be beneficial if researchers could agree on using a common set or a few sets of reference chemicals in their studies and if some organization would take the scientific responsibility for aiding in the development of a more integrated strategy.

Murk AJ, Legler J, Denison MS, Giesy JP, Van De Guchte C, Brouwer A. **Chemical-activated luciferase gene expression (CALUX): a novel in vitro bioassay for Ah receptor active compounds in sediments and pore water**. Fundam Appl Toxicol 1996;33(1):149-60.
This study demonstrates that the novel in vitro CALUX (chemical-activated luciferase expression) assay is a rapid, sensitive assay for assessing the toxic potency of (mixtures of) aryl hydrocarbon receptor (AhR)-active compounds in sediments and pore waters. A rat hepatoma (H4IIE) cell line, stably transfected with a construct containing the dioxin-responsive element (DRE) sequence and the luciferase reporter gene, was used to determine the relative potency or the total activities of AhR-active compounds in sediment and pore water extracts. This novel CALUX assay had a detection limit of 0.5 fmol of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The sensitivity and linear working range was slightly better than for the ethoxyresorufin O-deethylase (EROD) assay in H4IIE wild type cells. The primary improvement of the CALUX assay compared to the EROD assay, however, is that the CALUX assay is insensitive to substrate inhibition. The CALUX activity induced by organic extracts from 450-mg aliquots of sediment or 250-microl aliquots of pore water corresponded with the instrumentally analyzed degree of pollution of the sediment. Using pore water, only a simple and rapid extraction procedure was needed, without additional clean-up to prevent cell death. The response from pore water samples in an 8-day early life stage test with zebra fish (Branchydanio rerio) corresponded with the CALUX induction, although the correlation was sometimes disturbed by heavy metals. Two polychlorinated terphenyl mixtures, the PCB-substitute Ugilec 141, polybrominated diphenylethers, and the PCB-mixture Clophen A50 were tested in the CALUX assay and had induction potencies that were 10(-4)-10(-7) compared to TCDD.

GENOTOXICITY AND MUTAGENESIS

Aaron CS, Zimmer DM, Harbach PR, Yu RL. **End points for biomonitoring: assay sensitivity/selectivity**. Environ Health Perspect 1996;104(Suppl 3):521-5.
Estimation of population exposure and biological impact of potential hazards are central reasons for performing biomonitoring. The sensitivity of the biomonitoring methods and the linkage of the measured phenomenon to human disease are also important, but often overlooked, considerations. We are conducting experiments to evaluate the sensitivity of hprt mutation measurement in the nonhuman primate, the cynomolgus monkey. Our findings demonstrate in the monkey that hypoxanthine guanine phosphoribosyltransferase (hprt) mutations produced in vivo can be detected using technique originally worked out using human cells; cynomolgus monkeys were chosen to avoid many of the complications encountered in studying humans. Sequencing of mutants from the monkey using reverse transcriptase polymerase chain reaction methods has led us to conclude that there is similarity of the spectra observed between the spontaneous mutations detected in the two species. However, more recent data suggest that due to low sensitivity, the method is probably not appropriate for routine biomonitoring of randomly selected populations. For example, the inability of the hprt mutation assay to detect some very potent mutagens in the monkey and the effects of the time-dependent pattern of mutant occurrence serve to urge caution in interpretation of elevation or lack of elevation in mutant frequency. Mechanisms for splitting and archiving samples of human tissues/blood from populations at risk may prove valuable as methods improve.

Adams K, Jones E, Takahashi N. **In vitro mutagenicity studies with toborinone (OPC-18790)**. Yakuri To Chiryo 1996;24(11):2339-46.
CBAC COPYRIGHT: CHEM ABS In vitro mutagenicity studies were performed in order to assess the mutagenic

and clastogenic potential of toborinone, a novel quinolinone deriv. and pos. inotropic agent. These tests were: a bacterial reverse mutation (Ames) test using Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, TA1538 and Escherichia coli WP2 uvr A; a chromosome aberration test on Chinese hamster ovary cells; a mammalian cell mutation assay on mouse lymphoma L5178Y cells. In all 3 studies, testing was carried out in both the absence and presence of exogenous metabolic enzyme systems (S-9 mix). In the bacterial reverse mutation study, no evidence of mutagenic potential of toborinone was obsd. In the chromosome aberration test some small but significant increases in chromosome aberration were seen, but these were not concn. dependent or reproducible. In the mammalian cell mutation assay some evidence of mutagenic potential was obsd. These results indicate that toborinone was mutagenic in the mammalian cell mutation assay. In the chromosome aberration test there was insufficient evidence to consider toborinone to be clastogenic, and the bacterial reversion study was completely neg.

Anard D, Kirsch-Volders M, Elhajouji A, Belpaeme K, Lison D. **In vitro genotoxic effects of hard metal particles assessed by alkaline single cell gel and elution assays**. Carcinogenesis 1997;18(1):177-84.
Hard metals (WC-Co) are made of a mixture of cobalt metal (Co, 5-10%) and tungsten carbide particles (WC, >80%). Excessive inhalation of WC-Co is associated with the occurrence of different lung diseases including an excess of lung cancers. The elective toxicity of hard metal is based on a physico-chemical interaction between cobalt metal and tungsten carbide particles to produce activated oxygen species. The aim of the present study was to assess the genotoxic activity of hard metal particles as compared with Co and WC alone. In human peripheral lymphocytes incubated with Co or WC-Co, a dose- and time-dependent increased production of DNA single strand breaks (ssb) was evidenced by alkaline single cell gel electrophoresis (SCGE) and modified alkaline elution (AE) assays. Addition of 1 M formate, a hydroxyl radical scavenger, had a protective effect against the production of ssb by both WC-Co or Co alone. On the basis of an equivalent cobalt-content, WC-Co produced significantly more ssb than Co. WC alone did not produce DNA ssb detectable by the AE assay, but results obtained with the SCGE assay may suggest that it either allows some uncoiling of the chromatin loops or induces the formation of slowly migrating fragments. Overall, this in vitro study is the first demonstration of the clastogenic property of cobalt metal-containing dusts. The results are consistent with the implication of an increased production of hydroxyl radicals when Co is mixed with WC particles. The SCGE results also suggest that WC may modify the structure of the chromatin, leading to an increased DNA sensitivity to clastogenic effects. Both mechanisms are not mutually exclusive and may concurrently contribute to the greater clastogenic activity of WC-Co dust. This property of WC-Co particles may account for the excess of lung cancers observed in hard metal workers.

Anderson D, Blowers SD, Marrs TC, Rice P. **An in vitro and an in vivo unscheduled DNA synthesis assay with a zinc oxide/hexachloroethane (Zn/HCE) smoke**. Hum Exp Toxicol 1996;15(1):38-44.
BIOSIS COPYRIGHT: BIOL ABS. 1. Since Zn/HCE smoke has been shown previously to be weakly positive in the Ames test, and negative in the bone marrow micronucleus assay, other assays including a second in vivo assay examining unscheduled DNA synthesis (UDS) in rat hepatocytes has been carried out, as recommended by the UK Department of Health guidelines. 2. Zn/HCE smoke was assessed for its ability to induce DNA repair in an UDS assay both in vitro in cultured rat hepatocytes and in rat hepatocytes after in vivo treatment by inhalation. 3. For the in vitro investigation, two studies were carried out assessing media exposed to Zn/HCE smoke using at least seven concentrations up to a toxic level. At the highest concentration of Zn/HCE smoke, where some viable cells were seen, an increase in UDS was observed in both experiments. However this was not statistically significant, was only seen at a level where toxicity was observed and was therefore considered not to be biologically significant. 4. In the in vivo investigation, one study was carried out in three separate parts, assessing two doses of Zn/HCE smoke characterised by their zinc content as approximately 20 and 56 mug l-1 air. A dose-related increase in UDS was observed which was not statistically significant. The positive control behaved as anticipated, showing a highly statistically significant response. It was concluded that Zn/HCE smoke did not induce unscheduled DNA repair in the in vitro or in vivo UDS assays under the conditions used in the studies. The overall lack of genotoxic effect of this smoke in this and previous studies in this lab. would not suggest a major health hazard.

Arriaga-Alba M, Flores-Paz R, Diaz-Hernandez R, Gonzalez-Patino ME. [**Assessment of genetic toxicity of the**

exposure to clomiphene citrate, with various bacterial test systems]. Ginecol Obstet Mex 1996;64:490-7. (Spa)
Clomiphene citrate (CC) induces framshift mutations on the Salmonella typhimurium Ames strains TA1538, TA97 and TA100 employing in vitro metabolic activation with S9 aerochlor 1254 induced rat livers, but not base pair substitution mutations with neither the standard or the preincubation method. CC induced genolethal DNA damages on the Escherichia coli PolA-/PolA+ with S9 on the preincubation method or without S9 on the disk diffusion one. The severe primary DNA damages produced b CC was verified by the SOS induction on the lysogenic lambda phage induction with De Marini (1988) method and the induction of colicin E1 plasmid on E. coli. These results are suggestive that CC may be an adduct forming compound which is able to inhibit replication if the cell lacks DNA polymerase, or it may produce framshift mutations after replications. CC induced damages could be large lesions conducing to unicatenary DNA strains, that are able to induce the lexA regulated genes. So, the use of this ovulation inductor is a risk of genotoxic damage and it is advisable to do a risk-benefit evaluation in any particular case before its prescription.

Avent ND, Martin PG, Armstrong-Fisher SS, Liu W, Finning KM, Maddocks D, Urbaniak SJ. **Evidence of genetic diversity underlying Rh D-, weak D (Du), and partial D phenotypes as determined by multiplex polymerase chain reaction analysis of the RHD gene**. Blood 1997;89(7):2568-77.
CBAC COPYRIGHT: CHEM ABS The human blood group Rh antigens are expressed by proteins encoded by a pair of highly homologous genes located at chromosome 1p34-36. One of the genes (RHCE) encodes Rh CcEe antigens, while the other (RHD) the D antigen. Point mutations in the RHCE gene generate the C/c and E/e polymorphisms, while it has been shown that an RHD gene deletion can generate the D-neg. phenotype. We have analyzed intron 4 of the RHCE and RHD genes and have defined the site of an RHD-specific deletion located in this intron. Using a multiplex RHD typing assay, which combines a reverse polymerase chain reaction (PCR) primer, which straddles this RHD-specific sequence, and a pair of primers located in exon 10 of the RHD gene, we have analyzed 357 different genomic DNA samples derived from individuals expressing D+, D-, weak D, and partial D phenotypes. Of these, we have noted a significant discordance with our multiplex PCR assay in the D- phenotypes dCcee and dccEe (which have been previously described) and weak D phenotypes. Our results suggest that in five serol. D- individuals we have identified an apparently intact RHD gene. Sequence anal. of transcripts obtained from one of these individuals (of phenotype dCCee) illustrates the presence of full-length RHD transcripts, which have a point mutation at nucleotide 121 (C .fwdarw. T), which generates an in-frame stop codon (Gln41Stop). Thus, we describe a different mol. basis for generating the D- phenotype to the complete RHD gene deletion described previously. We also show that there are discordances with serotype and the multiplex assay in weak D and partial D phenotypes, indicating that the underlying mol. basis can be heterogeneous. Existing Rh D PCR assays assume the complete absence of the RHD gene in D-phenotypes.

Botta A, Occhipinti P, Bartfai E, Orsiere T. **Genotoxic effects of potassium dichromate in cultural human lymphocytes**. Med Sci Res 1996;24(12):797-9.
BIOSIS COPYRIGHT: BIOL ABS. Chromium derivatives are used in the steel industry. The trivalent form is the most common form but the hexavalent forms are of greater industrial importance. An impressive amounts of information is available regarding the genotoxicity of chromium derivatives. The hexavalent form can penetrate the cell whereas the trivalent form produced by metabolically reduction binds with nucleic acids. Chromium derivatives have not been tested in the cytokinesis-blocked micronucleus assay (CBMA). The present study compared the results obtained in a chromosome aberration test and in the CBMA.

Bunger J, Stork J, Stalder K. **Cyto- and genotoxic effects of coordination complexes of platinum, palladium and rhodium in vitro**. Int Arch Occup Environ Health 1996;69(1):33-8.
The growing industrial use of platinum group elements as catalysts, especially in automobile exhaust detoxification (trimetal catalytic converters), is causing increasing occupational and environmental pollution. The cytotoxic and mutagenic properties of industrially used coordination complexes of platinum, palladium and rhodium were investigated using the neutral red cytotoxicity assay on two established cell lines and the Salmonella typhimurium/ microsome test system (Ames test). Cytotoxic effects of the platinum complexes, measured as ED50, occurred at test concentrations of 0.2 mM. The analogous palladium salts tested were 3 times less toxic with ED50 being 0.6

mM, while the rhodium salts proved to be 30 times less toxic (ED50 = 6 mM). Levels of toxicity of the different complexes of a particular metal did not differ significantly from each other, which indicates that the metal itself is responsible for the toxic effects. In the Ames test, the spontaneous mutation rates increased by factors of 3 to 20 when the four tester strains were exposed to the platinum complexes. The analogous rhodium compounds proved to be considerably less mutagenic, and palladium demonstrated no mutagenic potential. As all of the four tester strains contain different mutations, the mutagenic potential of platinum and rhodium complexes appears to be based on a variety of mechanisms that damage DNA. From these in vitro experiments, it can be concluded that water-soluble complex salts of rhodium are less toxic and have a smaller mutagenic potential than the analogous platinum complexes. For palladium there is no evidence of any mutagenic property. From this point of view, the development of a catalytic converter containing predominantly palladium may be a possible means of minimizing potential health risks from this exhaust detoxification technique.

Chan F, Robinson J, Brownlie A, Shivdasani RA, Donovan A, Brugnara C, Kim J, Lau B, Witkowska HE, Zon LI. **Characterization of adult alpha- and beta-globin genes in the zebrafish**. Blood 1997;89(2):688-700.
CBAC COPYRIGHT: CHEM ABS Developmental switching of Hbs (Hbs) occurs in most vertebrates, yet the cellular and mol. basis for this process remains elusive. The zebrafish is a new genetic and developmental system that can be used to study embryogenesis, and mutants with a variety of defects in hematopoiesis have recently been derived. To initiate our studies on Hb switching in this organism, we have characterized the globins expressed in the adult. Reversed-phase high performance liq. chromatog. and mass spectrometric analyses of adult peripheral blood hemolyzates showed that there are three major alpha globins and two beta globins in circulating erythroid cells. In addn., we have isolated and characterized zebrafish adult alpha- and beta-globin cDNA clones that encode some of these globins. High levels of alpha- and beta-globin gene expression were detected in adult erythroid cells, whereas embryonic erythroid cells expressed little, if any, of these RNAs. We have also shown that the alpha- and beta-globin genes are tightly linked on the same chromosome and are arrayed in a 3'-5' to 5'-3' configuration, resp. The characterization of these genes and regulatory elements in this globin locus will provide insight into the process of globin gene transcription. With these reagents, future studies of Hb switching in zebrafish mutants with defective hematopoiesis will be possible.

Chetelat AA, Albertini S, Gocke E. **The photomutagenicity of fluoroquinolones in tests for gene mutation, chromosomal aberration, gene conversion and DNA breakage (Comet assay)**. Mutagenesis 1996;11(5):497-504.
The ability of fluoroquinolones to cause light-induced adverse effects has been established in experimental studies and clinical observations. The formation of active oxygen species appears to be responsible for this activity. Photomutagenicity tests with bacterial, lower eukaryotic and mammalian cells were performed with three fluoroquinolones (Fleroxacin, Ciprofloxacin and Lomefloxacin). After concomitant irradiation with simulated solar light (with a reduced UVB component), weak increases in the number of revertants were observed in Salmonella typhimurium TA104 and TA100. No photomutagenic activity was detected in Saccharomyces cerevisiae D7. In the chromosomal aberration (CA) test with Chinese hamster V79 cells the number of aberrant metaphases was markedly increased. In the Comet assay with mouse lymphoma cells, evidence of extensive DNA breakage was obtained. All three compounds showed similar potencies in the Comet and Ames assays while there was a clear gradation of potencies in the CA assay (Lomefloxacin > Fleroxacin > Ciprofloxacin), which conformed with reports on the relative potencies regarding phototoxicity. The oxygen radical scavengers catalase, superoxide dismutase and N, N'-dimethylurea modulated the photoclastogenicity and phototoxicity but had no influence on the effects in the Comet and Ames tests. It thus appears that different kinds of mechanism are responsible for toxicity and clastogenicity on the one side and DNA breakage and gene mutation.

Collas P, Alestrom P. **Rapid targeting of plasmid DNA to zebrafish embryo nuclei by the nuclear localization signal of SV40 T antigen**. Mol Mar Biol Biotechnol 1997;6(1):48-58.
CBAC COPYRIGHT: CHEM ABS Binding SV40 T antigen nuclear localization signals (NLSs) to plasmid DNA promotes transgene expression following injection of DNA-NLS complexes into the cytoplasm of zebrafish eggs. We now demonstrate that NLS peptides mediate import of DNA from the cytoplasm into embryo nuclei, under

conditions in which naked DNA is not imported. Plasmid DNA was localized by polymerase chain reaction (PCR) in isolated nuclei, and relative amts. were quantified by densitometry. Binding DNA to NLSs, but not to nuclear-import-deficient peptides, promoted rapid targeting of DNA-NLS complexes to nuclei, and transport across the nuclear envelope. Import of DNA-NLS complexes was competed by co-injected albumin-NLS conjugates. NLS, but not reverse NLS, was detected on blots of nuclei probed with 32P-labeled DNA. The results suggest that NLS-mediated DNA transfer into nuclei may constitute a valuable tool for several gene transfer applications.

Consuegra S, Ferreiro JA, Maria Sierra L, Comendado MA. **'Non-genotoxic' carcinogens evaluated using the white-ivory assay of Drosophila melanogaster**. Mutat Res 1996;359(2):95-102.
An attempt was made to assess carcinogens which were nongenotoxic by the Ames test but gave positive results on the yeast DEL system, which measures induction of intrachromosomal recombination, using the Drosophila-melanogaster white/ivory (w(i)) assay. The chemicals evaluated included acetamide (60355), thioacetamide (62555), safrole (94597), urethane (51796), amitrole (61825), ethionine (67210), methionine (59518) and auramine-O (492808), which were used in chronic and surface treatment assays. In the chronic treatment, 40 Drosophila couples were kept for 48 hours in bottles with a solution of the tested compound at concentrations ranging from 0.1 to 80 millimolar (mM). In the surface treatment, eggs from 60 couples were kept in bottles, to which a chemical solution at up to 800mM was added to the medium 48 hours later. The number of emerged adults from each treatment was compared to its corresponding control, and toxicity was evaluated. Reversion frequency was estimated by observation of revertant spots. Of the chemicals, only urethane gave a positiveresponse in the w(i) assay. The authors conclude that, despite what is indicated by the yeast DEL system, most of the chemicals do not induce intrachromosomal recombination in D-melanogaster.

Cunningham ML, Hayward JJ, Shane BS, Tindall KR. **Distinction of mutagenic carcinogens from a mutagenic noncarcinogen in the big blue transgenic mouse**. Environ Health Perspect 1996;104(Suppl 3):683-6.
The aromatic amines 2,4-diaminotoluene (2,4-DAT) and 2,6-diaminotoluene (2,6-DAT) are structural isomers that have been extensively studied for their mutagenic and carcinogenic characteristics. Both compounds are rapidly absorbed after oral administration and are equally mutagenic in the Ames test; however, 2,4-DAT is a potent hepatocarcinogen, whereas 2,6-DAT does not produce an increased incidence of tumors in rats or mice at similar doses. The Big Blue transgenic B6C3F1 mouse carries multiple copies of the lacI mutational target gene. Our studies were designed to determine whether the Big Blue system could be used to detect differences in the vivo mutagenic activity between the carcinogen-noncarcinogen pair 2,4-DAT and 2,6-DAT and to determine whether the in vivo mutagenesis assay results correspond to the rodent carcinogen bioassay results. Male B6C3F1 transgenic mice were exposed to 2,4-DAT or 2,6-DAT at 0 or 1,000 ppm in the diet for 30 and 90 days or to dimethylnitrosamine as a positive control. Mutant frequencies were nearly identical for all three groups at 30 days, while at 90 days the mutant frequency for the hepatocarcinogen 2,4-DAT (12.1 +/- 1.4 x 10(-5)) was significantly higher ($p < 0.01$) as compared to bouth age-matched (spontaneous) controls (5.7 +/- 2.9 X 10(-5)) and the 2,6-dat-exposed group (5.7 +/- 2.4 X 10(-5)). Results from this study demonstrate that the big blue transgenic mutation assay can distinguish differences in vivo between the mutagenic responses of hepatic carcinogens and a noncarcinogen; is sensitive to mutagens through subchronic dietary exposure; and yields a differential response depending upon the length of time mice are exposed to a mutagen.

Currie PD. **Zebrafish genetics: mutant cornucopia**. Curr Biol 1996;6(12):1548-52.
The initial characterization of mutations from the large-scale mutagenesis of the zebrafish genome has been reported. What new insights will we gain about vertebrate development from these studies?

Dayan AD. **Transgenic rodents in toxicology**. Int J Exp Pathol 1996;77(6):251-6.

De Bertoldi M. **Genotoxic effects of pesticides**. Eur J Cancer Prev 1996;5(5):397-9.

Delaney CA, Eizirik DL. **Intracellular targets for nitric oxide toxicity to pancreatic beta-cells**. Braz J Med Biol Res 1996; 29(5):569-79.
The radical nitric oxide (NO) may be a mediator of pancreatic beta-cell damage in early insulin-dependent diabetes mellitus (IDDM). Under the stimulus of cytokines, invading macrophages and the beta-cell themselves may produce large amounts of NO, leading to beta-cell dysfunction and death. It still remains to be determined which are the intracellular targets for NO-induced damage. Available data from rat islets indicate that the radical inactivates the mitochondrial enzyme aconitase, impairing substrate oxidation and ATP production. Ionic channels and complexes I and II of the mitochondrial electron transport chain are two other possible targets for NO effects which may impair insulin secretion. NO also leads to nuclear DNA damage in both rat and human pancreatic beta-cells, as evaluated by the 'comet assay'. The effects of NO at the DNA level are complex, and involve formation of N-nitrosoamines, deamination of purines and pyrimidines, or damage induced by peroxynitrite. Besides inducing over DNA damage. NO may also inactivate DNA repair/replication enzymes. The outcome of NO-induced beta-cell DNA damage can be cell death by apoptosis or, in some cases, necrosis. Upon cell damage beta-cells trigger cell repair mechanisms. This seems also to be the case following NO exposure, and insulin-producing cells are able to regain their function following treatment with non-lethal concentrations of NO. A better understanding of the mechanisms involved in NO-induced beta-cell damage and repair may be instrumental in developing new strategies for IDDM prevention.

Dennog C, Hartmann A, Frey G, Speit G. **Detection of DNA damage after hyperbaric oxygen (HBO) therapy**. Mutagenesis 1996;11(6):605-9.
Hyperbaric oxygen (HBO) therapy is successfully used for the treatment of a variety of conditions. However, exposure to high concentrations of oxygen is known to induce damage to cells, possibly due to an increased oxygen radical production. As reactive oxygen species also cause DNA damage, we investigated the DNA-damaging effect of HBO with the alkaline version of the single cell gel test (comet assay). Oxidative DNA base modifications were determined by converting oxidized DNA bases to strand breaks using bacterial formamidopyrimidine-DNA glycosylase (FPG), a DNA repair enzyme, which specifically nicks DNA at sites of 8-oxo-guanines and formamidopyrimidines. HBO treatment under therapeutic conditions clearly and reproducibly induced DNA damage in leukocytes of all test subjects investigated. Increased DNA damage was found immediately at the end of the treatment, while 24 h later, no effect was found. Using FPG protein we detected significant oxidative base damage after HBO treatment. DNA damage was detected only after the first treatment and not after further treatments under the same conditions, indicating an increase in antioxidant defences. DNA damage did not occur when the HBO treatment was started with a reduced treatment time which was then increased stepwise.

Director AE, Nath J, Ramsey MJ, Swiger RR, Tucker JD. **Cytogenetic analysis of mice chronically fed the food mutagen 2-amino-1-methyl-6-phenylimidazo(4,5b)pyridine**. Mutat Res 1996;359(1):53-61.
BIOSIS COPYRIGHT: BIOL ABS. The cytogenetic effects in mice chronically fed the heterocyclic amine 2-amino-1-methyl-6-phenylimidazo(4,5b)pyridine (PhIP) were evaluated by chromosome painting, micronucleated normochromatic erythrocytes (MN NCEs) and sister chromatid exchanges (SCEs). PhIP and numerous other heterocyclic amines have been isolated from cooked foods, and many have been found to be carcinogenic in laboratory rodents. Female C57BL/6N mice were chronically fed a diet containing 0, 100, 250 or 400 ppm of PhIP beginning at 8 weeks of age. Peripheral blood and bone marrow were taken from 5 mice per treatment group at 1, 4 and 6 months from the start of exposure. PhIP was removed from the diet for a final month of the experiment, at which time blood was taken from the remaining animals. Chromosome-specific composite DNA probes for mouse chromosomes 2 and 8 were hybridized to metaphase cells from each tissue. The 1- and 4-month time points showed no statistically significant difference between the control and exposed mice for either tissue in chromosome aberration frequencies. Both MN NCEs and SCEs were analyzed at a single time point during exposure (4 months for MN NCEs and 6 months for SCEs) and again 1 month after removing PhIP from the diet. MN NCEs in the peripheral blood showed a statistically significant dose response, with all values decreasing significantly I month after removing PhIP from the diet. SCE frequencies in the peripheral blood showed an approximate doubling compared to control mice, and decreased to control levels 1 month after removing PhIP from the diet. SCE frequencies in the bone marrow of exposed mice showed no difference from the control animals. These results show that chronic ingestion of PhIP by female C57BL/6 mice does not produce persistent cytogenetic damage as

visualized by chromosome aberrations, MN NCEs or SCEs.

Donovan PJ, Smith GT, Lawlor TE, Cifone MA, Murli H, Keefer LK. **Quantification of diazeniumdiolate mutagenicity in four different in vitro assays**. Nitric Oxide 1997;1(2):158-66.
CBAC COPYRIGHT: CHEM ABS Diazeniumdiolates are under investigation as possible prodrugs of the multifaceted bioregulatory agent nitric oxide. This study was undertaken to assess further the mutagenic potential of two diazeniumdiolates, DEA/NO (Et2N[N(O)NO]Na) and SPER/NO ([H2N(CH2)3NH(CH2)4N[N(O)NO-](CH2)3NH +3]), which generate NO spontaneously with half-lives at 37.degree. and pH 7.4 of 2 and 39 min, resp. The genotoxic potential of these compds. was investigated with the Ames bacterial reverse mutation assay, two mammalian cell gene mutation assays (CHO/HGPRT and L5178Y TK+/-), and an assay for sister chromatid exchange (SCE) using Chinese hamster ovary (CHO) cells. Both diazeniumdiolates had previously been shown to be mutagenic in the Ames Salmonella plate assay. In the expts. reported here, Salmonella typhimurium strain TA1535 was exposed to the compds. in a liq. incubation assay for either 15 min or 48 h without an S-9 fraction. With the 15-min exposure, DEA/NO was mutagenic at concns. of 0.625 mM (3.5 .times. control) and greater, while SPER/NO was mutagenic at 0.5 mM (2.7 .times. control) and above. In the CHO/HGPRT assay, DEA/NO was weakly mutagenic only at the highest concn. used, 20 mM, inducing a mutant frequency per survivor that was 2.5 . times. control, while SPER/NO was mutagenic at 0.5 mM with a mutant frequency of 2.5 .times. control. When the CHO cells were given 10 repetitive 20 mM DEA/NO exposures (3 min each), HGPRT mutant frequency was 4.1 . times. control. In the L5178Y mouse lymphoma cell TK+/- assay, DEA/NO doubled the mutation rate at 1.82 mM, while SPER/NO's mutation frequency was more than twice that of control at 0.63 mM. DEA/NO was pos. in the SCE assay without metabolic activation, yielding significant SCE at 1.25, 2.5, and 5 mM that was 1.8, 2.2, and 2.6 times control, resp. SPER/NO increased the SCE by 1.2, 1.4, and 1.3 times at 1.5, 2.0, and 2.5 mM. The results suggest that the two diazeniumdiolates, although mutagenic in the bacteria, are much weaker mutagens in mammalian cells.

Douglas GR, Gingerich JD, Soper LM, Jiao J. **Toward an understanding of the use of transgenic mice for the detection of gene mutations in germ cells**. Mutat Res 1997;388(2-3):197-212.
Recently-developed transgenic models have provided unprecedented access to rodent somatic and germ line tissues for the study of gene mutation in vivo. While mutations in germ cells are considered an important aspect of any regulatory assessment of the risks posed by chemicals, currently-available conventional tests, which involve the study of thousands of offspring make it impractical to test large numbers of chemicals, for the induction of inherited gene mutations. When effects in germ cells per se, rather than offspring are acceptable targets, transgenic mouse assays may provide a practical alternative. As part of an international collaborative study to begin to determine the reliability, efficacy, and role of such assays, lacZ transgenic mice (Muta Mouse) were treated with single i.p. doses of ethylnitrosourea (ENU), methyl methanesulfonate (MMS), and isopropyl methanesulfonate (iPMS), and mutant frequencies determined using phenyl-beta-D-galactoside (p-gal) positive selection. For studies using germ cells, the selection of sampling times and target cells is crucial. Spermatagonial stem cells and cells in post-spermatagonial stem cell stages are the critical target cell populations of regulatory importance. Cell populations within these categories were studied by sampling germ cells isolated from seminiferous tubules and spermatozoa from the epididymis at 91 days and 25 days after treatment. The data show that ENU and iPMS induced mutations in post-spermatagonial stem cells and spermatagonial stem cells. However, MMS did not induce mutations in either cell type, or at either sampling time, at doses approaching lethality. This result is possibly because MMS induces preferentially large lesions and chromosomal aberrations (as opposed to point mutations), which are not readily detectable with bacteriophage-based shuttle vectors. Since MMS-induced specific locus and dominant lethal mutations are induced only after the mid-spermatid stage, it is also possible that the timing used missed this effect. While the ENU and iPMS data in this study demonstrate the suitability of the lacZ male transgenic mice for the study of gene mutations in post-spermatagonial stem cells and spermatagonial stem cells by sampling cells isolated from seminiferous tubules at selected times after treatment, the MMS results do not answer fully whether transgenic mouse mutation assays can detect mutations resulting from lesions induced after the mid-spermatid stage when most cellular processing is retarded. Nevertheless, it appears clear from presently available information, that the bacteriophage-based lacZ transgenic model is suitable for the detection of gene mutations in spermatogonial stem cells, spermatocytes, and early spermatids.

Dugan AM, Barilyak IR. [**Assessment of mutagen activity of gas air particles by means of the microbiological test**]. Mikrobiol Zh 1995;57(4):66-72. (Rus)
BIOSIS COPYRIGHT: BIOL ABS. Total mutagen activity of a gas component in chemical pollutions of the atmospheric air in a number of industrially developed towns of Ukraine has been studied and assessed in a microbiological test on Salmonella tiphimurium. The towns were chosen proceeding from the specificity of industry: metallurgic industry (Mariupol, Zaporozhie, Donetsk, Krivoj Rog, Makeevka); chemical industry (Cherkassy, Chernigov, Kremenchug, Severodonetsk, Lisichansk, Gorlovka, Povno, Sumy) and conditionally control ones (Simferopol, Sevastopol, Nikolaev, Poltava, Zhitomir). The air samples, 100 m3, have been taken in each town weekly during a month by special absorbers of POLYSORB-2 type. The extraction of chemical matters from absorbers was carried out by traditional methods. Chemical matters were dissolved in dimethyl sulphoxide and Lested for its ability to induce the gene mutations. The studies have shown that the atmospheric air samples from the group of metallurgic towns prove the mutagen activity, classified as the middle one (the number of revertant colonies in the experiment exceeded the control 10.3 to 22.2 times). The mutagenicity of chemical towns was on the level of middle and weak, that of conditionally control ones was on the level of weak only.

Duthie SJ, Collins AR. **The influence of cell growth, detoxifying enzymes and DNA repair on hydrogen peroxide-mediated DNA damage (measured using the comet assay) in human cells**. Free Radic Biol Med 1997;22(4):717-24.
Single-cell gel electrophoresis (the comet assay) is a sensitive method for detecting strand breaks at the level of individual cells. Cells embedded in agarose are lysed, electrophoresed, and fluorescently stained. Breaks in the DNA release its supercoiling and allow DNA to extend toward the anode, resembling a comet. We have used the comet assay to investigate the influence of growth state, xenobiotic detoxifying enzymes, and DNA repair processes on the response of cultured human cells to oxidative damage. HepG2 and Caco-2 cells are differentiated liver and colon cell lines, respectively. HeLa and GM1899A cells are relatively unspecialized epithelial and lymphoblastoid cells. Substrate-dependent cells showed a cyclical fluctuation of glutathione (GSH) with respect to growth. Enzyme activities (glutathione reductase, glutathione peroxidase, and catalase) varied considerably between cell types and changed with cell growth state. Hydrogen peroxide induced more DNA damage in actively dividing cells than in confluent cultures. Sensitivity to oxidative injury did not correlate with detoxifying enzyme activity. Rather, differences in susceptibility between cells could be correlated with differences in DNA repair capacity. This study highlights the need to standardize experimental conditions if the comet assay is to be employed in the study of genotoxicity.

Easton M Dl, Kruzynski GM, Solar II, Dye HM. **Genetic toxicity of pulp mill effluent of juvenile Chinook Salmon (Onchorhynchus tshawytscha) using flow cytometry**. Water Sci Technol 1997;35(2-3):347-55.
BIOSIS COPYRIGHT: BIOL ABS. On-site bioassays were conducted at the furthest upstream pulp mill on the Fraser River in British Columbia. Uncontaminated river water was used to dilute treated effluent as discharged from the final diffuser pond. A single cohort of juvenile (8-10 gm) chinook salmon (Oncorhynchus tshawytscha) was divided into an hypoxic group receiving 65% oxygen saturated water and a normoxic group receiving ambient 88% oxygen saturated water. Both groups were exposed over a period of 30 days to effluent concentrations of 2%, 4%, 8%, and 16%, while the controls received uncontaminated river water. This range of concentrations spanned those encountered by wild juvenile salmon overwintering in the upper Fraser River mainstem. The blood when analyzed by flow cytometry showed significant concentration-dependent clastogenic damage in both the nontoxic and hypoxic groups starting at the 4% concentration. A concentration-response curve was determined from the hypoxic data set. Genetic implications of mutagenic damage to natural populations of chinook salmon are discussed along with the utility of the flow cytometer in detecting genotoxic damage.

Ehrenberg L, Granath F, Tornqvist M. **Macromolecule adducts as biomarkers of exposure to environmental mutagens in human populations**. Environ Health Perspect 1996;104(Suppl 3):423-8.
A cancer epidemiologist recently said that adduct measurement has so far been of little use to epidemiological research. This remark gives us a starting point for the discussion of the purposes of measuring macromolecule

adducts that originate from electrophilic compounds or metabolites in humans and animals. Historically, methods for adduct monitoring were developed as a means of determining target doses that, combined with measurements of genotoxic potencies, could be used for risk assessment. With mass spectrometric methods, adducts can be quantified at levels that are thousands of times lower than those in which the cancer incidence associated with this exposure is detectable in disease-epidemiological studies. Furthermore, mass spectrometric techniques permit identification of the chemical structure of the adduct, particularly in the case of hemoglobin adducts. Adduct measurement therefore constitutes not only a means of risk estimation but it may be used as a complement of disease epidemiology in situations in which, for statistical reasons, the risk is too low to be detectable--which does not signify that the risk is acceptably low. It also gives a possibility of identification of the dangerous components in mixed exposures and of the relevant reactive intermediates in cases of complex metabolism.

Endo O, Sekiya Y, Koyano M, Seki Y, Goto S, Matsushita H. [**A comparison of extraction methods for mutagens in urine. Sep-Pak tC18 and blue rayon**]. Jpn J Toxicol Environl Health 1996;42(5):429-32. (Jpn) BIOSIS COPYRIGHT: BIOL ABS. A comparison of two extraction methods (Sep-Pak and blue rayon) of mutagens in the urine was demonstrated using the salmonella mutagenicity test. Most of the urine extracts with Sep-Pak tC18 were more mutagenic than those with blue rayon. It was suggested that the Sep-Pak extraction method was useful for the evaluation of human exposure to carcinogens/mutagens in daily life based on the mutagenic activity of the urine.

Foresti M, Paoletti I, Mele F, Geraci G. **Two periods of sensitivity to mutagens in induced Mel cells with different outcomes**. Mutat Res 1997;374(2):269-75.
Mouse erythroleukemia (Mel) cells are particularly sensitive to mutagenic agents between 18 and 24 h from the start of induction (Foresti, M.L. Gaudio, G. Geraci and P. Manduca (1986) Inhibition of dimethyl sulfoxide induced erythropoietic differentiation of murine erythroleukemia cells in culture. Cancer Res., 46, 6260-6263). We show here the occurrence of another period of sensitivity during the initial 5 h after the addition of the inducer dimethyl sulfoxide (DMSO) to the culture medium. The sensitivity to the mutagenic action of a sublethal 3-s pulse of UV light (13.5 J/m2) was monitored on the progeny of the irradiated cells at day 5 after the start of induction. The effects were analysed on functions strictly linked to the final expression of the differentiated phenotype: hemoglobin concentration, percent cells producing hemoglobin (%B+), activity of delta-amino levulinic acid dehydrase (ALA-DH) and presence of globins. Each function appeared differently and selectively affected in the progeny of the cells depending on the exact time of irradiation during the period of sensitivity Specifically, cells irradiated at hour 3 after induction show both hemoglobin concentration and ALA-DH activity values increased by a factor 3 over controls. Cells irradiated at hour 5 show an almost complete halt in cell induction and the other tested functions show minimal values. Cells are nearly insensitive to irradiation at later times, until hour 20, after which a second period of sensitivity with peak value at hour 22 occurs at which time hemoglobin concentration in the progeny of irradiated cells is increased by a factor 3 over controls, ALA-DH activity is increased by a factor 15 while percent B+ value is at its minimum. The differential effects of UV irradiation on Mel cell functions in the first and in the second period of sensitivity to mutagens confirm the hypothesis that the consequences of a mutational event are strictly dependent on the functional state of the cell. The 1-5 h period of sensitivity in which Mel cells fix the effects of the mutagen in their genome corresponds to increased thymidine incorporation not correlated with cell duplication.

Fretland AJ, Doll MA, Gray K, Feng Y, Hein DW. **Cloning, sequencing, and recombinant expression of NAT1, NAT2, and NAT3 derived from the C3H/HeJ (rapid) and A/HeJ (slow) acetylator inbred mouse: functional characterization of the activation and deactivation of aromatic amine carcinogens**. Toxicol Appl Pharmacol 1997;142(2):360-6. An acetylator polymorphism has been described in the mouse and the inbred strains C3H/HeJ and A/HeJ constitute rapid and slow acetylators, respectively. The NAT1, NAT2, and NAT3 genes from C3H/HeJ and A/HeJ acetylator inbred mouse strains were amplified using the polymerase chain reaction, cloned into the plasmid vector pUC19, and sequenced. They were then subcloned into the prokaryotic expression vector pKK223-3 and expressed in Escherichia coli strain JM105. The 870-bp nucleotide coding region of NAT1 and NAT3 did not differ between the rapid and slow acetylator mouse strains, or from that of previously published mouse NAT1 and NAT3 sequences. However, NAT2 did differ between the rapid and slow acetylator strains with an A296 T transition

which causes a (Asn99-->Ile) substitution in the deduced amino acid sequence. Recombinant NAT1, NAT2, and NAT3 proteins catalyzed N-, O-, and N,O-acetyltransferase activities. NAT3 catalyzed aromatic amine N-acetyltransferase activities at very low rates, which confirms a previous study. Apparent K(m) and Vmax kinetic constants for N-acetylation were 5- to 10-fold lower for recombinant mouse NAT1 than NAT2. Intrinsic clearances for recombinant mouse NAT1- and NAT2-catalyzed N-acetylation of aromatic amine carcinogens were comparable. Both recombinant.

Fritz A, Rozowski M, Walker C, Westerfield M. **Identification of selected gamma-ray induced deficiencies in zebrafish using multiplex polymerase chain reaction**. Genetics 1996;144(4):1735-45.
The ease with which mutations can be generated in zebrafish makes this vertebrate an important resource for developmental genetics and genome studies. We have developed a PCR-based screening method that allows the efficient identification of gamma-ray induced deficiencies targeted to selected sequences. We describe three mutants characteristic of our findings and show that these mutations include deletions and translocations that can affect as much as 1% of the genome. These deficiencies provide a basis for analyzing the functions of cloned zebrafish genes using noncomplementation screens for point mutations induced by high-efficiency chemical mutagenesis.

Gaspar J, Laires A, Va S, Pereira S, Mariano A, Quina M, Rueff J. **Mutagenic activity of glycine upon nitrosation in the presence of chloride and human gastric juice: a possible role in gastric carcinogenesis**. Teratog Carcinog Mutagen 1996;16(5):275-86.
The mutagenic activity of glycine upon nitrosation was studied in the Ames tester strains TA98, TA100, TA102, and TA104. The results obtained show that glycine at acidic pH values and in the presence of Cl- can react with nitrite giving rise to genotoxic compounds to the tester strains used. When these experiments were carried out in the presence of gastric juice the genotoxicity observed was associated with the Cl- concentration in the different gastric juice samples. The nature and the mechanism of genetic lesion induced by the ultimate genotoxicant arising from the nitrosation of glycine are not fully understood. Primary amines (e.g., amino acids) have been described as potential alkylating agents after nitrosation. However, in our experimental conditions these alkylating activities were not detected, suggesting that other mechanisms could be involved in the genetic lesion induced by nitrosated glycine. The influence of Cl- in the genotoxic activity of glycine and other primary amines upon nitrosation and its possible involvement in the etiology of gastric cancer are discussed.

Gebel T, Lantzsch H, Plessow K, Dunkelberg H. **Genotoxicity of platinum and palladium compounds in human and bacterial cells**. Mutat Res 1997;389(2-3):183-90.
Platinum and palladium belong to the group of platinum elements and thus share many chemical properties. Platinum coordination complexes are known to be carcinogenic and genotoxic in mammalian and bacterial cells. However, little is known about palladium genotoxicity. This study compares and evaluates the genotoxic potential of selected platinum and palladium metal salts in mammalian and bacterial cells using the cytokinesis-block micronucleus test (MNT) with human lymphocytes and the bacterial SOS chromotest. Carboplatin, cisplatin(II), transplantin(II), PtCl4(IV), and K2PtCl4(II) caused a significantly elevated genotoxicity in the MNT and the SOS chromotest. The platinum compounds PtCl2(II) and K2PtCl6(IV), and the divalent palladium salts PdCl2(II), K2PdCl4 (II), Pd(NH3)2J2(II), Pd(NH3)4Cl2(II), and transpalladium(II) were not genotoxic in the MNT nor in the SOS chromotest. Therefore, evidence for palladium genotoxicity seems to be low in mammalian and bacterial cells.

Gelsthorpe M, Pulumati M, Mccallum C, Dang-Vu K, Tsubota SI. **The putative cell cycle gene, enhancer of rudimentary, encodes a highly conserved protein found in plants and animals**. Gene 1997;186(2):189-95.
CBAC COPYRIGHT: CHEM ABS The enhancer of rudimentary gene, e(r), in Drosophila melanogaster encodes a protein, ER, whose function has been implicated in pyrimidine biosynthesis and the cell cycle (Wojcik et al. (1994) Genetics 138, 1163-1170). In order to identify conserved regions of the protein and potentially important functional domains, the e(r) gene was cloned and sequenced from two other insects (Drosophila virilis and Aedes aegypti) and three vertebrates (Homo sapiens, Mus musculus, and Brachydanio rerio) and sequenced from a flowering plant

(Arabidopsis thaliana). These sequences along with those of a nematode (Caenorhabditis elegans) exhibit a high degree of identity. ER of Drosophila melanogaster is 76 identical to the three vertebrate proteins, 49 identical to the nematode protein, and 40 identical to the plant protein. There is high evolutionary conservation among the vertebrates. The mouse and human proteins are identical and differ from that of the zebrafish by a single conservative amino-acid change (valine for isoleucine). A dramatic sequence conservation is seen in the position of the hydrophobic amino acids. Of the 27 positions occupied by hydrophobic amino acids in ER of Drosophila melanogaster, 25 of the corresponding positions in the human protein, 23 of the positions in Caenorhabditis elegans, and 20 of the positions in Arabidopsis thaliana have hydrophobic amino acids. Most of these residues are present in three conserved amphipathic alpha-helixes, which are proposed to function in protein-protein interactions. Two phosphorylation sites for casein kinase II (CKII) have also been conserved within the animal groups. Purified ER from Drosophila melanogaster is phosphorylated in vitro by CKII, arguing that these two sites are functional in vivo. A putative shift in the secondary structure of ER caused by the phosphorylation of these sites suggests that CKII may be regulating the activity of the ER in vivo.

Gorovaia AI, Bobyr' LF, Skvortsova TV, Digurko VM, Klimkina II. [**The methodological aspects of assessing the mutagenic background and genetic risk for man and the biota from the action of mutagenic ecological factors**]. Tsitol Genet 1996;30(6):78-86. (Rus)
Methodology of the assessment of territory state for mutagenic background and genetic risk was proposed. It is based on analysis of results of cytogenetic monitoring of high-sensitive bioindicators and children populations as well as an analysis of statistic data on hereditary and ecology-dependent diseases in human populations. Methodology has been tested under the conditions of Dnepropetrovsk Province, that allowed to specify areas of increased genetic risk.

Griffiths SD, Clarke AR, Healy LE, Ross G, Ford AM, Hooper ML, Wyllie AH, Greaves M. **Absence of p53 permits propagation of mutant cells following genotoxic damage**. Oncogene 1997;14(5):523-31.
Much evidence has been gathered in support of a critical role for p53 in the cellular response to DNA damage. p53 dysfunction is associated with progression and poor prognosis of many human cancers and with a high incidence of tumours in p53 knockout mice. The absence of a p53-dependent G1 arrest that facilitates DNA repair or apoptosis might impact critically on clinical cancer in two ways. First, by abrogating the impact on therapy that operates via genotoxic damage and apoptosis; and second, by encouraging progression either by inducing genomic instability and DNA mis-repair or by permitting survival of mutants. However, experiments examining the relationship between p53 deficiency and mutation frequency have so far failed to confirm these predictions. The precise role played by p53 is therefore unclear. We now report use of a short term in vitro approach to assess the influence of p53 on radiation-induced mutations at the hprt locus in murine B cell precursors that are normally radiation ultrasensitive. We find a high number of hprt mutants among X-irradiated p53 null cells, which results from preferential survival as clonogenic mutants rather than from a p53-dependent increase in mutation rate. This result has important implications for genotoxic cancer therapy.

Han JS. **Mutagenic activity and specificity of hydrogen peroxide in the ad-3 forward-mutation test in two-component heterokaryons of Neurospora crassa**. Mutat Res 1997;374(2):169-84.
In the ad-3 forward-mutation test, hydrogen peroxide was at best a weak mutagen in nongrowing conidia from a DNA repair-proficient heterokaryon (H-12, uvs-2+/uvs-2+) but was a moderate mutagen in nongrowing conidia from a DNA-repair-deficient heterokaryon (H-59, uvs-2/uvs-2) over a narrow range of high concentrations. H-59 also was more sensitive than H-12 to the killing activity of hydrogen peroxide at high concentrations. Thus, a DNA-repair pathway, of which the gene product of the uvs-2+ allele is a part, appears to be involved in the repair of hydrogen peroxide-induced DNA lesions at low survival in these strains. There was slightly, but significantly, more killing by hydrogen peroxide of nongrowing conidia from H-12 and H-59 in the presence of O2 than in the absence of O2 (presence of N2). Thus, the killing activity of hydrogen peroxide was enhanced by O2. The Mutational Spectra of hydrogen peroxide-induced ad-3 mutants shows that hydrogen peroxide induced mainly gene/point mutations but also some multilocus deletion mutations in H-12 and H-59. Multiple-locus mutations occurred only in H-59, but the frequency was very low. The frequencies of the 3 kinds of intracistronic complementation pattern among ad-3BR

mutants (gene/point mutations) suggest that hydrogen peroxide induced both base-pair substitutions and frameshift mutations in both strains.

Hanelt S, Helbig R, Hartmann A, Lang M, Seidel A, Speit G. **A comparative investigation of DNA adducts, DNA strand breaks and gene mutations induced by benzo[a]pyrene and (.+-.)-anti-benzo[a]pyrene-7,8-diol 9,10-oxide in cultured human cells**. Mutat Res 1997;390(1-2):179-88.
CBAC COPYRIGHT: CHEM ABS Genotoxic effects of benzo[a]pyrene (BP) and its reactive metabolites (.+-.)-anti-benzo[a]pyrene-7,8-diol 9,10-oxide ((.+-.)-anti-BPDE) were comparatively investigated in vitro with the permanent human fibroblast cell line MRC5CV1. Induced DNA adducts were measured by 32P-postlabeling, DNA strand breakage was detd. by the comet assay and the HPRT gene mutation test was used to detect cytotoxicity and mutagenicity. Treatment of MRC5CV1 cells with S9 mix-activated BP or with (.+-.)-anti-BPDE resulted in a concn.-dependent increase in DNA adducts and strand breaks. Genotoxic effects of BP and (.+-.)-anti-BPDE were detected by 32P-postlabeling and the comet assay with similar sensitivity.

Hannigan MP, Cass GR, Penman BW, Crespi CL, Lafleur AL, Busby WFJ, Thilly WG. **Human cell mutagens in Los Angeles air**. Environ Sci Technol 1997;31(2):438-47.
BIOSIS COPYRIGHT: BIOL ABS. The human cell mutagenicity of particulate air pollution samples collected in southern California is measured. The human cell mutation assay used in this study tests mutagenic activity at the thymidine kinase locus in h1A1v2 cells using a 72-h exposure. Throughout 1993, airborne fine particle samples were taken at a regional background site on San Nicolas Island upwind of Los Angeles and at four urban sites: Long Beach, central Los Angeles, Azusa, and Rubidoux. The Long Beach site is in close proximity to direct emissions from industrialized sources including power plants, petroleum refineries, and the Los Angeles-Long Beach harbor complex. Central Los Angeles was chosen because of its dense vehicle traffic, railroad yards, and proximity to the central business district. Azusa and Rubidoux are photochemical smog receptor sites located generally downwind of the highest density of primary emissions sources. No systematic seasonal variation of the mutagenic potency (mutagenicity per unit organic aerosol mass) is observed at any of the urban sites. This suggests that the important human cell mutagens are not dominated by a seasonal emission source such as wood combustion and that if the atmospheric transformation products of photochemical air pollution are involved, then these reactions must occur during the winter as well as during the summer photochemical smog season. No significant spatial variation of annual average mutagenic potency of the aerosol was observed between three of the four urban sites; while the average mutagenic potency of the Long Beach aerosol was slightly higher than elsewhere in the air basin. This similarity of mutagenic potency values across widely separated monitoring sites suggests that the mutagenicity of the aerosol is due largely to ubiquitous emission sources (e.g., motor vehicle traffic or stationary source fuel combustion) rather than to proximity to isolated point sources of unusual mutagenic organics. The mutagen concentration per cubic meter of ambient air was computed by weighing the mutagenic potency values of the aerosol according to the mass concentration of organics present at each monitoring site. The human cell mutagen concentration in Los Angeles urban air was found to be 1 order of magnitude greater than at the background site studied upwind of the city, showing that the city is indeed a source of human cell mutagens.

Hartmann A, Speit G. **The contribution of cytotoxicity to DNA-effects in the single cell gel test (comet assay)**. Toxicol Lett 1997;90(2-3):183-8.
We evaluated genotoxic and cytotoxic effects of the three non-mutagenic and non-carcinogenic compounds p-nitrophenol, D-menthol and sodium N-lauroyl sarcosine which have previously been shown to induce DNA double strand breaks (DNA dsb) secondary to induced cytotoxicity. We tested whether genotoxic effects in the alkaline single cell gel test (comet assay) may be confounded by cytotoxicity-induced DNA dsb. Cell viability was determined at the end of the treatment using the fluorescein diacetate/ethidium bromide-assay and plating efficiency was used as an indicator of long-term survivability. Experiments with V79 Chinese hamster cells and human white blood cells revealed negative results in the comet assay despite strong cytotoxic effects. However, cells with extremely fragmented DNA ('clouds') occurred but were excluded from the evaluation under the principle that they represent dead cells. We also noticed a significant loss of cells at cytotoxic concentrations that might be attributed to the induction of highly fragmented DNA which is lost during electrophoresis. Since the comet assay

allows the determination of DNA effects on the single cell level, a confounding effect of cytotoxicity on test results can be avoided.

Hei TK, Wu LJ, Liu SX, Vannais D, Waldren CA, Randers-Pehrson G. **Mutagenic effects of a single and an exact number of alpha particles in mammalian cells**. Proc Natl Acad Sci U S A 1997;94(8):3765-70.
One of the main uncertainties in risk estimation for environmental radon exposure using lung cancer data from underground miners is the extrapolation from high- to low-dose exposure where multiple traversal is extremely rare. The biological effects of a single alpha particle are currently unknown. Using the recently available microbeam source at the Radiological Research Accelerator Facility at Columbia University, we examined the frequencies and molecular spectrum of S1- mutants induced in human-hamster hybrid (A(L)) cells by either a single or an exact number of alpha particles. Exponentially growing cells were stained briefly with a nontoxic concentration of Hoechst dye for image analysis, and the location of individual cells was computer-monitored. The nucleus of each cell was irradiated with either 1,2,4, or 8 alpha particles at a linear energy transfer of 90 keV/microm consistent with the energy spectrum of domestic radon exposure. Although single-particle traversal was only slightly cytotoxic to A(L) cells (survival fraction approximately 0.82), it was highly mutagenic, and the induced mutant fraction averaged 110 mutants per 10(5) survivors. In addition, both toxicity and mutant induction were dose-dependent. Multiplex PCR analysis of mutant DNA showed that the proportion of mutants with multilocus deletions increased with the number of particle traversals. These data provide direct evidence that a single a particle traversing a nucleus will have a high probability of resulting in a mutation and highlight the need for radiation protection at low doses.

Honma M, Hayashi M, Sofuni T. **Cytotoxic and mutagenic responses to X-rays and chemical mutagens in normal and p53-mutated human lymphoblastoid cells**. Mutat Res 1997;374(1):89-98.
To investigate the role of p53 as a guardian of the genome, the mutagenic and cytotoxic responses to mutagens were compared for normal (TK6) and p53-mutated (WTK-1) cells. The characteristics of the mutations that occurred in these cells was also examined. Human lymphoblastoid cell lines TK6 and WTK-1 are derived from the same progenitor cell line, but WTK-1 cells have homozygous p53 mutations resulting in overproduction of mutant p53 protein. The spontaneous mutation frequency at the heterozygous thymidine kinase (tk) locus in TK6 and WTK-1 cells was 3.5 X 10(-6) and 101.1 X 10(-6), respectively. WTK-1 cells were more resistant than TK6 cells to cytotoxic damage by X-rays, ethyl methanesulfonate (EMS) and methyl methanesulfonate (MMS), and were more sensitive at the tk locus to the mutagenic effects of X-rays, EMS, MMS and mitomycin C. Molecular analysis of TK mutants by Southern-hybridization demonstrated that 70% of spontaneous mutations and 86% of X-ray induced mutations in TK6 cells resulted from loss of the entire tk allele (loss of heterozygosity; LOH), while 95% of spontaneous and 100% of X-ray induced mutations showed LOH in WTK-1 cells. Densimetric analysis revealed that almost all of the LOH mutants in WTK-1 cells were homozygous at the tk locus, consistent with inter-allelic homologous recombination, or gene conversion. These data indicate that p53-mutated WTK-1 cells are hypermutable, susceptible to some environmental mutagens, and prone to LOH-type gene mutations because of their abnormally high recombinational activity. It may be that genetic instability in p53-mutated cells significantly contribute to the subsequent occurrence of LOH mutations during a multistep tumorigenic process.

Hundal BS, Dhillon VS, Sidhu IS. **Genotoxic potential of estrogens**. Mutat Res 1997;389(2-3):173-81.
A genotoxicity evaluation of three commonly used estrogens-ethinyl estradiol, cyclotriol and cyclodiol was undertaken using short-term in vitro and in vivo assays. None of the drugs caused significantly increased or decreased number of His+ mutants to appear in the Ames Salmonella assay, either with or without S9 mix or in a modified host-mediated version of this assay. However, the clastogenic potential of these drugs became evident from the increased number or chromosome aberrations and sister chromatid exchanges (SCEs) induced by these drugs in human lymphocyte cultures both in the presence and absence of S9 mix. Increased frequencies of micronuclei and of sister chromatid exchanges in mice confirmed their clastogenic potential.

Jacobson-Kram D, Tepper J, Kuo P, San RH, Curry PT, Wagner VO, Putman DL. **Evaluation of potential genotoxicity of pulsed electric and electromagnetic fields used for bone growth stimulation**. Mutat Res

1997;388(1):45-57.
Medical devices emitting pulsed electric and electromagnetic fields have been found to be effective for a number of clinical applications including stimulation of bone and tissue growth. To determine whether pulsed fields of the type used in these clinical applications present a mutagenic hazard, electric and electromagnetic fields at two exposure levels were tested in the Ames test, CHO cell chromosomal aberration assay, BALB/3T3 cell transformation assay and unscheduled DNA synthesis assay in primary rat hepatocytes. For both field types, initial and independent repeat studies were performed for each assay at both clinical and supra clinical doses. In all assays, the results show a lack of cytotoxic, transforming and mutagenic activity. The data suggest that pulsed electric and electromagnetic fields of the type and dose levels used in bone growth stimulation lack mutagenic and transforming activity.

Johnsen NM, Schwarze PE, Nyholm SH, Lag M, Becher R, Brunborg G, Holme JA. **Genotoxic effects of cyclopenta-fused polycyclic aromatic hydrocarbons in different types of isolated rat lung cells**. Carcinogenesis 1997;18(1):193-9.
The genotoxic effects of the environmental contaminants benz[j]aceanthrylene (B[j]A), benz[l]aceanthrylene (B[l]A) and benzo[a]pyrene (B[a]P), and the metabolism of radiolabelled B[j]A, were studied using rat lung microsomes and various types of isolated rat lung cells from control and Aroclor 1254 (PCB) treated animals. All three compounds (10 or 20 microg/plate) resulted in low, but detectable, levels of His+ revertants in the Salmonella assay when plated with control lung microsomes. The two cyclopenta polycyclic aromatic hydrocarbons (CP-PAH) B[j]A and B[l]A, gave increased levels of revertants when plated with microsomes from PCB-treated animals. Clara cells, type 2 cells and alveolar macrophages isolated from control rats were exposed to B[j]A, B[l]A or B[a]P (30 microg/ml, 1 h), but neither of the cell types showed any DNA damage when measured by alkaline filter elution. However, both B[j]A and B[l]A (30 microg/ml, 2 h) caused DNA adducts in all three cell types, measured by the 32P-post-labelling technique, whereas no B[a]P adducts were detected (30 microg/ml, 2 h). The total DNA adduct levels in Clara cells, type 2 cells and macrophages exposed to B[j]A were 0.085 +/- 0.033, 0.053 +/- 0.001 and 0.170 +/- 0.030 fmol/microg DNA, respectively, whereas the total levels in cells exposed to B[l]A were 0.140 +/- 0.070, 0.140 +/- 0.030 and 0.220 +/- 0.080 fmol/microg DNA, respectively. Cells exposed to B[j]A revealed only one adduct which corresponds with the B[j]A-1,2-oxide DNA adduct. Judged from high performance liquid chromatography (HPLC) analysis using radiolabelled B[j]A (30 microg/ml, 30 min), the major metabolite formed in control microsomes was B[j]A-1,2-diol. Thus, oxidation at the cyclopenta ring appears to be the most important activation pathway for B[j]A with control rat lung cells. Exposure of lung cells to CP-PAH (30 microg/ml, 2 h) isolated from PCB pretreated rats resulted in slightly increased DNA adduct levels in Clara cells and macrophages when compared to cells isolated from control rats. Furthermore, the adduct pattern had shifted, and no apparent B[j]A-1,2-oxide adduct could be detected on the thin layer chromatography (TLC) plate. In contrast, the major metabolite formed with microsomes from PCB-treated animals was still the B[j]A-1,2-diol.

Kang MY, Choi YH, Nam SH. [**Screening of antimutagenic activities from cereals and beans including rice**]. Han'Guk Nonghwa Hakhoechi 1996;39(6):419-23. (Kor)
CBAC COPYRIGHT: CHEM ABS We have established the quant. method for assay antimutagenic activity from natural products using SOS chromotest technique. Establishment of the method in this study makes it possible to det. antimutagenic activities from samples in term of the sample amt. required for 50% inhibition to mutagenic activity induced by the chem. mutagen under the std. assay condition. Antimutagenic activities of rice from different cultivars as well as other cereals were assayed through this method. The results revealed that antimutagenic activities of mutant cultivar, Suwon 393 (Hyangdo) and Sanghaehyanghyulla (Jado), were higher than Chuchung which is mainly consumed for steamed rice, and also indicated that antimutagenic activities of cereals, such as job's tear, buckwheat, small red bean, black bean were generally higher than that of brown rice.

Kasamatsu T, Kohda K, Kawazoe Y. **Synergetic cytotoxicity of bleomycin and polyhydric alcohols: DNA strand breakage evaluated by comet assay**. Biol Pharm Bull 1996;19(4):632-5.
DNA strand breakage induced in cultured cells by bleomycin (BLM) was remarkably enhanced in the presence of glycerol or polyvinyl alcohol which produces an enhancement effect on BLM cytotoxicity, but not in the presence of

alcohols such as methanol, ethanol, or polyethylene glycol which do not produce enhanced cytotoxicity. The comet assay, a useful analytical method for evaluating DNA strand breakage, clearly demonstrated that combinations of BLM and polyhydric alcohols induced serious DNA damage in the whole cell populations compared with those induced by treatment with BLM alone. The comet assay also successfully showed distributions of cell populations with various degrees of DNA damage. These results suggested that an increase in DNA damage induced in the presence of polyhydric alcohols might be responsible for the enhancement of BLM cytotoxicity.

Katagiri T, Hirono I, Aoki T. **Identification of a cDNA for medaka cytoskeletal beta-actin and construction for the reverse transcriptase-polymerase chain reaction (RT-PCR) primers**. Fish Sci 1997;63(1):73-6.
CBAC COPYRIGHT: CHEM ABS A full-length cDNA for medaka cytoskeletal beta-actin was isolated from a liver cDNA library. The entire coding region was 1125 bp long and was well conserved with respect to other vertebrate beta-actins. Two primers for a reverse transcriptase-polymerase chain reaction (RT-PCR) assay were constructed from the nonvariable region among medaka, carp, chicken, and mouse beta-actin. RT-PCR amplified a 510 bp DNA band from medaka cDNA synthesized from the blood, brain, heart, intestine, kidney, liver, muscle, ovary, spleen, and testis poly(A)+mRNA. The constructed primers were used successfully to detect a specific 510 bp fragment of beta-actin from zebrafish, rainbow trout, and Japanese flounder.

Katoh M, Horiya N, Valdivia RP. **Mutations induced in male germ cells after treatment of transgenic mice with ethylnitrosourea**. Mutat Res 1997;388(2-3):229-37.
The present study was undertaken to clarify whether the transgenic mouse mutagenesis assay system can be used instead of dominant lethals or specific locus test after treatment of male germ cells in mouse with ethylnitrosourea (ENU). Male Big Blue transgenic mice (BB) carrying a lacI target gene were given a single intraperitoneal injection of 150 mg/kg ENU. Vasa deferential sperm, caudal epididymal sperm or whole testes were assayed for mutation at 3, 14, 22 and 93 days after treatment with ENU. The average of background lacI- mutant frequencies was 2.05 x 10 (-5). The MF observed in post spermatogonial stage after treatment with ENU were slightly increased over background. On the other hand, ENU induced high MF in the spermatogonial stage. MF detected after treatment of BB male germ cells with ENU were lower than those detected in the mouse visible specific-locus mutations in previous reports. Nevertheless, it is clear that this assay is a practical alternative to the specific locus test for detecting mutations induced in spermatogonial stage.

Katsifis SP, Kinney PL, Hosselet S, Burns FJ, Christie NT. **Interaction of nickel with mutagens in the induction of sister chromatid exchanges in human lymphocytes**. Mutat Res 1996;359(1):7-15.

Kevekordes S, Grahl K, Zaulig A, Dunkelberg H. **Nitro musk compounds. Genotoxic activity. Genotoxicity testing of nitro musks with the SOS-chromotest and the sister-chromatid exchange test**. Environ Sci Pollut Res Int 1996;3(4):189-92.
CBAC COPYRIGHT: CHEM ABS Musk xylene, ketone, ambrette, moskene, and tibetene were tested for genotoxic activity. Nitro musks revealed no genotoxicity neither in the SOS chromotest with E. coli PQ37 nor in the sister-chromatid exchange test with human lymphocytes.

Knapik EW, Goodman A, Atkinson OS, Roberts CT, Shiozawa M, Sim CU, Weksler-Zangen S, Trolliet MR, Futrell C, et al . **A reference cross DNA panel for zebrafish (Danio rerio) anchored with simple sequence length polymorphisms**. Development 1996;123:451-60.
CBAC COPYRIGHT: CHEM ABS The ultimate informativeness of the zebrafish mutations described in this issues will rest in part on the ability to clone these genes. However, the genetic infrastructure required for the positional cloning in zebrafish is still in its infancy. Here we report a ref. cross panel of DNA, consisting of 520 F2 progeny (1040 meioses) that has been anchored to a zebrafish genetic linkage map by 102 simple sequence length polymorphisms. This ref. cross DNA provides: (1) a panel of DNA from the cross that was used to construct the genetic linkage map, upon which polymorphic gene(s) and genetic markers can be mapped; (2) a fine order mapping tool with a max. resoln. of 0.1 cM; and (3) a foundation for the development of a phys. map (an ordered

array of clones each contg. a known portion of the genome). This ref. cross DNA will serve as a resource enabling investigators to relate genes or genetic markers directly to a single genetic linkage map and avoid the problem of integrating different maps with different genetic markers, as must be currently done when using randomly amplified polymorphic DNA markers, or as has occurred with human genetic linkage maps.

Lantzsch H, Gebel T. **Genotoxicity of selected metal compounds in the SOS chromotest**. Mutat Res 1997;389 (2-3):191-7.
Knowledge concerning the genotoxicity of inorganic metal compounds in the SOS chromotest is limited. Up to now, only Cr(VI), Sn(II) and the platinum antitumor compound cisplatin(II) were shown to be genotoxic in this test system. However, for Cr(VI) and Sn(II), a positive reaction could only be achieved in cytotoxic dose ranges. The aim of the present study was to provide additional data concerning metal salt genotoxicity in the SOS chromotest. Therefore, 14 metal/metalloid salt compounds of platinum, palladium, rhodium, arsenic, antimony and chromium were tested. Four platinum salts, K2PtCl4, cis-Pt(NH3)2Cl2 (cisplatin), trans-Pt(NH3)2Cl2 (transplatin) and PtCl4 as well as two rhodium compounds tested, K2RhCl5 and (NH4)3RhCl6, could be shown to be genotoxic in the chromotest using the tester strain Escherichia coli PQ37. A moderate genotoxicity was shown by the two Cr(VI) compounds K2CrO4 and K2Cr2O7. All palladium compounds and all the other metal salts tested were unable to induce a significant SOS response.

Leavitt J, Fatone M, Hestdalen C, Obringer JW, Tillinghast HS Jr. **Mutagenic activity of high-energy 532 nm ultra-short laser pulses**. Radiat Res 1997;147(4):490-4.
The mutagenic activity of green (532 nm) and infrared (1064 nm) ultra-short laser light pulses was tested in cultured Syrian hamster fibroblasts by a hypoxanthine phosphoribosyl-transferase (HPRT) mutagenesis assay. In 18 irradiation trials, cells were exposed to eight consecutive 100-ps pulses of either 532 nm or 1064 nm light from a Nd: YAG laser at average irradiances of 3.0 GW/cm2. The 532 nm irradiations produced Hprt mutations at an average observed frequency of 5.3-5.6 x 10(-6), 10-fold higher than control trials ($P < 0.01$), while 1064 NM irradations produced only background (spontaneous mutation) frequencies. A HAT (Hypoxanthine, Aminopterin, Thymidine) sensitivity test allowed us to infer that HPRT-clones, seleceted as 6-thioguanine-resistant clones, possessed mutations at the HPRT locus after 532 NMND:YAG laser irradation. The mutagenic effects of 532 NM high-energy laser pulses and not 1064 NM wavelengths are discussed in light of a two-photon absorption hypothesis. These preliminary findings suggest that 460-590 NM visible-light lasers may be mutagenic to mammalian cells either as a result of two-photon absorption or through some other photochemical process that damages DNA.

Lee JJ, Trizna Z, Hsu TC, Spitz MR, Hong WK. **A statistical analysis of the reliability and classification error in application of the mutagen sensitivity assay**. Cancer Epidemiol Biomarkers Prev 1996;5(3):191-7.
In vitro mutagen hypersensitivity, determined with the bleomycin assay, has been found to be an independent risk factor for developing primary upper aerodigestive tract cancers, lung cancers, and second malignant tumors. The average number of chromatid breaks per cell (b/c) is derived from evaluating an arbitrarily set number of metaphases (usually 50) in each sample. The reliability of such an approach is of key importance in large-scale epidemiological studies. Because evaluating metaphases is a time-consuming task, it is desirable to know the minimum number of readings needed to reach an acceptable reliability. Statistical analyses were performed in 160 observations for which b/c values were determined by the same observer scoring 100 consecutive metaphases per sample. The b/c values were between 0.14 and 1.30 (mean, 0.61). The b/c values were separately calculated for the first and second sets of 50 metaphases. There was essentially no difference in the mean b/c values between the first and second 50 readings (difference, -0.002). The correlation between the two sets of readings was 0.72. The SE of the b/c values, based on scoring 50 metaphases, was 0.15. When scoring 100 metaphases, the SE decreased to 0.11. After evaluating the first 50 metaphases, the theoretical gain in reducing the SE was <1% with each additional reading. When 0.8 was used to dichotomize the mean B/C value into bleomycin-resistant or bleomycin-hypersensitive groups, sensitivity and specificity of reading 50 metaphases were above 75% and 95% when compared to 100 readings. A simulated case control study showed that there is a 15% attenuation in estimating the odds ratio with 50 readings. The findings suggest that the conventional methods of reading metaphases can yield acceptable reliability in our setting and may be applied to other cancer epidemiology studies.

Liegibel UM, Schmezer P. **Detection of the two germ cell mutagens ENU and iPMS using the LacZ/transgenic mouse mutation assay**. Mutat Res 1997;388(2-3):213-8.
BIOSIS COPYRIGHT: BIOL ABS. We have investigated the mutagenic effects of ENU, a potent mutagen in mouse spermatogonia, and of the two postmeiotic germ cell mutagens MMS and iPMS in germ cells, using a transgenic mouse mutation assay (lacZ/Muta Mouse, positive selection system). The test compounds were administered to 6-week-old animals by a single intraperitoneal injection. Seminiferous tubule germ cells were isolated from the testes after an expression time of 52 days and genomic DNA was extracted to examine induced mutations in the lacZ target gene. The spontaneous mutant frequencies observed in the control animals (n = 7) ranged from 3.5 to 17.90-5). ENU (150 mg/kg: n = 8) induced a 6.9-fold increase over controls, iPMS (100 mg/kg; n = 7) a 2.4-fold increase, and no effect at all was found following MMS treatment (80 mg/kg; n = 8). The study demonstrates that the transgenic mouse mutation assay is able to detect the germ cell mutagens ENU and iPMS in the target tissue. The critical steps of the assay, however, seem to be dosing and sampling time. In contrast, MMS has failed to induce germ cell mutations in seminiferous tubules of transgenic mice at the tested dose and after an expression period of 52 days.

Liu M, Grant SG, Macina OT, Klopman G, Rosenkranz HS. **Structural and mechanistic bases for the induction of mitotic chromosomal loss and duplication ('malsegregation') in the yeast Saccharomyces cerevisiae: relevance to human carcinogenesis and developmental toxicology**. Mutat Res 1997;374(2):209-31.
CBAC COPYRIGHT: CHEM ABS MultiCASE has the ability to automatically det. the structural features responsible for the biol. activity of chems. In the present study, 93 chems. tested for their ability to induce chromosomal 'malsegregation' in the yeast Saccharomyces cerevisiae were analyzed. This 'malsegregation' mimics mol. events that occur during human development and carcinogenesis resulting in an effective loss of one chromosome of an autosomal pair and duplication of the homolog. Structural features assocd. with the ability to induce such chromosome loss and duplication were identified and compared with those obtained from examn. of other toxicol. data bases. The most significant structural similarities were identified between the induction of chromosomal malsegregation and several toxicol. phenomena such as cellular toxicity, induction of sister chromatid exchanges in vitro and rodent developmental toxicity. Very significant structural similarities were also found with systemic toxicity, induction of micronuclei in vivo and human developmental toxicity. Less significant structural overlaps were found between yeast malsegregation and rodent carcinogenicity, DNA reactivity and mutagenicity, and the induction of chromosome aberrations in vitro and sister chromatid exchanges in vivo. These overlaps may indicate mechanistic similarities between the induction of chromosomal malsegregation and other toxicol. phenomena. The predictivity of the SAR model derived from the present data base is relatively low, however. This may be merely a reflection of the small size and compn. of the data base, however, further analyses suggest that it reflects primarily the multiple mechanisms responsible for the induction of chromosomal malsegregation in yeast and the complexity of the phenomenon.

Maddalena DJ, Snowdon GM. **Applications of genetic algorithms to drug design**. Expert Opin Ther Pat 1997;7 (3):247-54.
CBAC COPYRIGHT: CHEM ABS A review, with 51 refs., examg. the role of genetic and other evolutionary algorithms in the field of drug design. These methods have demonstrated their suitability in a variety of areas, including quant. structure-activity relationships (QSAR) and variable selection, conformation searching, receptor docking, receptor and pharmacophore elucidation, mol. de novo design and mol. selection with combinatorial libraries. Based upon the studies examd., genetic algorithms and other forms of evolutionary programs are likely to have a useful role in future drug design.

Maenhaut-Michel G, Janel-Bintz R, Samuel N, Fuchs RP. **Adducts formed by the food mutagen 2-amino-3-methylimidazo(4,5-f)quinoline induce frameshift mutations at hot spots through an SOS-independent pathway**. Molec Gen Genet 1997;253(5):634-41.
BIOSIS COPYRIGHT: BIOL ABS. The potency of 2-amino-3-methylimidazo(4, 5-f)quinoline (IQ) adducts to induce -

2, -1 and +1 frameshift mutations has been determined on specific target DNA sequences, namely short runs of alternating GpC sequences and short runs of guanines. The genetic control of the mutational processes has been analyzed using different Escherichia coli mutants, affected either in the control or in the mutagenesis pathway of the SOS system. We have shown that IQ adducts induce very efficiently both -1 and -2 frameshift mutations in E. coli. Both types of deletion mutations are induced in bacteria without the need of SOS induction, indicating that no LexA-controlled functions, in particular the UmuDC proteins, are required for mutation fixation. We have also shown that the frequency of IQ-induced -2 frameshift mutations in alternating GC sequences increases with the length of the repetition. The efficiency of IQ adducts to induce -1 and -2 frameshift mutations is similar to that of N-2-acetylaminofluorene (AAF) adducts. Both chemicals are potent carcinogens which form covalent adducts at the C8 position of guanines. We suggest that in both cases the adduct-induced DNA structure allows the replication complex to perform a mutagenic bypass of the lesion by a slippage mechanism. However, in contrast to AAF-induced frameshift mutagenesis, IQ-induced frameshift mutagenesis is SOS-independent.

Majtan V, Majtanova L. **Effect of some organic ammonium salts and amine oxides in the SOS chromotest**. Pharmazie 1996 Oct;51:753-5.

Marsteinstredet U, Brunborg G, Bjoras M, Soderlund E, Seeberg E, Kronberg L, Holme JA. **DNA damage induced by 3-chloro-4-(dichloromethyl)-5-hydroxy-2[5H]-furanone (MX) in HL-60 cells and purified DNA in vitro**. Mutat Res 1997;390(1-2):171-8.
CBAC COPYRIGHT: CHEM ABS We have investigated the induction of DNA damage by MX in a promyelocytic human leukemia cell line (HL-60 cells). Exposure of HL-60 cells to 100-300 muM MX resulted in increased levels of DNA single-strand breaks and/or alkali-labile sites (SSBs) as detected by alk. filter elution. When adding inhibitors of DNA break repair (AraC plus hydroxyurea), increased levels of DNA SSBs were obsd. at very low concns. (1-3 muM) of MX, as obsd. by both alk. filter elution and the single-cell gel electrophoresis assay. Increased DNA SSBs could also be obsd. if DNA repair inhibitors were added immediately after exposure to 10 muM MX, indicating that low concns. of MX cause a relatively stable modification of DNA that may be recognized and incised by DNA repair enzyme activities. Further studies with DNA break repair inhibitors indicated that HL-60 cells exposed to 10 muM MX for 1 h repaired 50% of their initial DNA damage during a 2-h period and the repair appeared to be complete at 22 h. Anal. of MX-treated DNA by sequencing methods indicated that MX preferentially reacts with guanines in DNA.

Matsui M, Matsui K, Kawasaki Y, Oda Y, Noguchi T, Kitagawa Y, Sawada M, Hayashi M, Nohmi T, Yoshihira K, et al. **Evaluation of the genotoxicity of stevioside and steviol using six in vitro and one in vivo mutagenicity assays**. Mutagenesis 1996;11(6):573-9.
Stevioside, a constituent of Stevia rebaudiana, is commonly used as a non-caloric sugar substitute in Japan. The genetic toxicities of stevioside and its aglycone, steviol, were examined with seven mutagenicity tests using bacteria (reverse mutation assay, forward mutation assay, umu test and rec assay), cultured mammalian cells (chromosomal aberration test and gene mutation assay) and mice (micronucleus test). Stevioside was not mutagenic in any of the assays examined. The aglycone, steviol, however, produced dose-related positive responses in some mutagenicity tests, i.e. the forward mutation assay using Salmonella typhimurium TM677, the chromosomal aberration test using Chinese hamster lung fibroblast cell line (CHL) and the gene mutation assay using CHL. Metabolic activation systems containing 9000 g supernatant fraction (S9) of liver homogenates prepared from polychlorinated biphenyl or phenobarbital plus 5,6-benzoflavone-pretreated rats were required for mutagenesis and clastogenesis. Steviol was weakly positive in the umu test using S.typhimurium TA1535/pSK1002 either with or without the metabolic activation system. Steviol, even in the presence of the S9 activation system, was negative in other assays, i.e. the reverse mutation assays using S.typhimurium TA97, TA98, TA100, TA102, TA104, TA1535, TA1537 and Escherichia coli WP2 uvrA/pKM101 and the rec-assay using Bacillus subtilis. Steviol was negative in the mouse micronucleus test. The genotoxic risk of steviol to humans is discussed.

Michael SF. **Thermostable ligase-mediated incorporation of mutagenic oligonucleotides during PCR**

amplification. Methods Mol Biol 1997;67:189-95.

Morita T, Asano N, Awogi T, Sasaki YF, Sato S, Shimada H, Sutou S, Suzuki T, Wakata A, Sofuni T, et al. **Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (groups 1, 2A and 2B): the summary report of the 6th collaborative study by CSGMT/JEMS MMS. Collaborative Study of the Micronucleus Group Test**. Mammalian Mutagenicity Study Group. Mutat Res 1997;389(1):3-122 .
To assess the correlation between micronucleus induction and human carcinogenicity, the rodent micronucleus assay was performed on known and potential human carcinogens in the 6th MMS/CSGMT collaborative study. Approximately 100 commercially available chemicals and chemical groups on which there was little or no micronucleus assay data were selected from IARC (International Agency for Research on Cancer) Groups 1 (human carcinogen), 2A (probable human carcinogen) and 2B (possible human carcinogen). As minimum requirements for the collaborative study, 5 male mice were treated by intraperitoneal injection or oral gavage once or twice with each chemical at three dose levels, and bone marrow and/or peripheral blood was analyzed. Five positives and 2 inconclusives out of 13 Group 1 chemicals, 7 positives and 5 inconclusives of 23 Group 2A chemicals, and 26 positives and 6 inconclusives of 67 Group 2B chemicals were found. Such low positive rates were not surprising because of a test chemical selection bias, and we excluded well-known micronucleus inducers. The overall evaluation of the rodent micronucleus assay was based on the present data combined with published data on the IARC carcinogens. After merging, the positive rates for Groups 1, 2A and 2B were 68.6, 54.5 and 45.6%, respectively. Structure-activity relationship analysis suggested that the micronucleus assay is more sensitive to the genetic toxicity of some classes of chemicals. Those to which it is sensitive consist of (1) aziridines and bis(2-chloroethyl) compounds; (2) alkyl sulfonate and sulfates; (3) acyl-type N-nitroso compounds; (4) hydrazines; (5) aminobiphenyl and benzidine derivatives; and (6) azo compounds. Those to which it is less sensitive consist of (1) dialkyl type N-nitroso compounds; (2) silica and metals and their compounds; (3) aromatic amines without other functional groups; (4) halogenated compounds; and (5) steroids and other hormones. After incorporation of structure-activity relationship information, the positive rates of the rodent micronucleus assay became 90.5, 65.2 and 60.0% for IARC Groups 1, 2A and 2B, respectively. Noteworthy was the tendency of the test to be more sensitive to those carcinogens with stronger evidence human carcinogenicity.

Mountcastle-Shah E. **Ventro-posterior morphogenesis in zebrafish (Danio rerio): mutational analysis, genetic characterization and genomic mapping**. Diss Abstr Int, B 1997:57(10):6050.

Nacci DE, Cayula S, Jackim E. **Detection of DNA damage in individual cells from marine organisms using the single cell gel assay**. Aquat Toxicol 1997;35(3-4):197-210.
CBAC COPYRIGHT: CHEM ABS The single cell gel (SCG) or comet assay is a simple method by which DNA damage is expressed as relative nuclear 'tail' length of gel-embedded cells following alk. electrophoresis. While potentially applicable to any cell type, lab. expts. were conducted to examine the utility of the SCG method for the detection of genotoxicity in cells of marine fish and invertebrates. Selected cells included those from flounder (Pleuronectes americanus) and oysters (Crassostrea virginica). In vitro exposures were used to optimize parameters and evaluate sensitivity, reproducibility and dose-responsiveness of the SCG method. In vivo exposures were used to examine the effects of factors such as intra-animal variability on low level DNA damage detection. In one exptl. series, individually identified oysters were repeatedly sampled to monitor DNA damage and recovery following in vivo exposure to genotoxicant-spiked sediment. Preliminary results suggest that the SCG may be a useful tool to monitor for genotoxic environmental exposures and investigate pollution-mediated health effects.

Natarajan AT, Boei JJ, Darroudi F, Van Diemen PC, Dulout F, Hande MP, Ramalho AT. **Current cytogenetic methods for detecting exposure and effects of mutagens and carcinogens**. Environ Health Perspect 1996;104 (Suppl 3):445-8.
Most mutagens and genotoxic carcinogens are efficient inducers of chromosomal alterations in exposed cells. Two important classes of aberrations, namely structural and numerical, are recognized and both types of aberrations are associated with congenital abnormalities and neoplasia in humans. These alterations can be easily detected and quantified in human peripheral blood lymphocytes. Conventional staining techniques can be used to detect these

aberrations; this technique was used to estimate absorbed dose in the case of a radiation accident in Goiania, Brazil. A recently introduced fluorescent in situ hybridization technique (FISH) using DNA probes has increased the sensitivity and ease of detecting chromosome aberrations, especially stable chromosome aberrations. This technique allows, to some extent, the estimation of absorbed radiation dose from past exposures. Numerical aberrations can be directly estimated in metaphases by counting the number of FISH-painted chromosomes. Micronuclei are formed by lagging chromosome fragments or whole chromosomes during the anaphase stage of cell division. The nature of micronuclei as to whether they possess a centromere can be determined either by CREST staining (calcinosis, Raynoud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia) or FISH with centromere-specific DNA probes. In several carcinogen-exposed populations, such as heavy smokers or people exposed to arsenic, aneuploidy appears to be more common than structural aberrations. In victims of radiation accidents, aneuploidy (hyperploidy) has been found to be common in addition to structural aberrations.

Navarrete MH, Carrera P, De Miguel M, De La Torre C. **A fast comet assay variant for solid tissue cells. The assessment of DNA damage in higher plants**. Mutat Res 1997;389(2-3):271-7.
The single-cell gel electrophoresis or comet assay, under high alkaline conditions, detects low levels of DNA damage. In it, broken DNA migrates from the nucleus to the anode providing images similar to comets. To adapt this assay to solid tissue cells, nuclei were directly obtained from Allium cepa L. roots. The surface of each single fresh sharply cut meristem was exposed to a small drop of 50 mM Sorensen buffer at pH 6.8, placed on a regular agarose-coated slide. By immediately adding low melting point agarose at 30 degrees C, nuclei resulted embedded in agarose. A final layer of this agarose ended the preparative steps. Conventionaly prepared leukocytes were used as a control. The treatment with detergent (lysis step of the conventional assay) proved to be unnecessary for the nude nuclei. A 20 min-long electrophoresis (at 0.65 V. cm-1, 230 mA and 10 degrees C) was more sensitive than a 10 min-long one for detecting the differential response of plant nuclei to 2 and 4 Gy of gamma-irradiation. A short fixation in methanol transformed the preparations into semi-permanent ones, without altering their later DNA staining by ethidium bromide. The use of instantaneously isolated nuclei simplifies and expands the use of this technique to any eukaryotic cell from solid tissues.

Ogheri S, Rampazzo C, Celotti L. **Mutagenic effects at hprt locus and in minisatellite sequences induced in V79 cells by treatments with UV and methyl-nitro-nitroso guanidine**. Mutat Res 1995;348(4):193-9.
BIOSIS COPYRIGHT: BIOL ABS. DNA alterations induced in V79 cells treated with UV light or methyl-nitro-nitrosoguanidine were analyzed by the mutagenicity test at the hprt locus and by DNA fingerprint analysis. Treated and control cells were seeded in the presence or absence of 6-thioguanine to determine mutant frequency and cell survival. From clonal cultures of the same cell populations we isolated a number of clones and grew them up individually to obtain appropriate amounts of DNA. High molecular weight DNA was digested with HinfI or HaeIII and hybridized with 32P-labelled 33.15 multilocus probe. The induction of 6-thioguanine resistant cells depended on the mutagen dose. The highest value of mutant frequency obtained was 7475 27 muM), corresponding to 0.7 percent of clonable cells. DNA fingerprint analysis carried out on the same treated cells showed that DNA rearrangements occurred at minisatellites much more frequently than in transcribed sequences. UV irradiation produced the highest frequency of variation, modifying minisatellite patterns in about 50 percent of the analyzed clones.

Peitl P Jr, Sakamoto-Hojo ET, Colus I Md. **Genotoxic activity of the insecticide nuvacron (Monocrotophos) detected by the micronucleus test in bone marrow erythrocytes of mice and in CHO cells**. Braz J Genet 1996;19(4):571-6.
BIOSIS COPYRIGHT: BIOL ABS. The organophosphorus insecticide Nuvacron (Monocrotophos) is a very toxic agent widely utilized in Brazilian agriculture. To evaluate the clastogenic potential of this insecticide, in vivo and in vitro micronucleus (MN) assay experiments were carried out on Swiss mice and on Chinese hamster ovary (CHO) cells, respectively. Nuvacron administered at doses of 2.5 and 5.0 mg/kg induced a statistically significant increase in the frequencies of MN detected in polychromatic bone marrow erythrocytes from animals (six/group) treated ip 24 h before. Exponentially growing CHO cells were treated continuously (16 h) with Nuvacron out using the cytokinesis-block method and a total of 6000 binucleated cells were scored to determine MN with 1 and 10 mug/ml Nuvacron. A

marked decrease in cell proliferation rates was observed for CHO cultures treated with higher concentrations. These data demonstrate that Nuvacron has a genotoxic effect on both in vivo and in vitro mammalian test systems.

Pesheva MG, Chankova SG, Avramova TS, Milanov DV, Genova GK. [**Genotoxic effect of cadmium chloride in various test-systems**]. Genetika 1997;33(2):183-8. (Rus)
Many industrial regions of Bulgaria are contaminated with cadmium. Induction of various genetic damages by four concentrations of cadmium chloride was studied in various test systems. None of the tested concentrations induced gene mutations in Salmonella typhimurium. An increase in frequency of gene mutations, mitochondrial mutations, and intragene recombination was detected in Saccharomyces cerevisiae treated with the highest cadmium chloride concentration. A clastogenic effect and a significant decrease in mitotic index (MI) were induced in radicle meristem cells of Pisum sativum L. by the two highest cadmium chloride concentrations. Cadmium chloride was also shown to increase the frequency of sex-linked recessive lethals (SLRLs) and dominant lethals (DLs) in Drosophila germ cells. The results obtained in different test systems allow cadmium chloride to be considered a weak mutagen inducing various genetic damages.

Phillips DH. **DNA adducts in human tissues: biomarkers of exposure to carcinogens in tobacco smoke**. nviron Health Perspect 1996;104(Suppl 3):453-8.
Tobacco smoking causes millions of cancer deaths annually. Tobacco smoke is a complex mixture of thousands of chemicals including many known animal carcinogens. Because many carcinogens from DNA adducts in target animal or human tissues, the detection of the formation of adducts using such methods as postlabeling, immunoassay, fluorescence spectroscopy, and mass spectrometry is a means of monitoring human exposure to tobacco carcinogens. Smokers are at increased risk of cancer in many organs, and studies have revealed either specific adducts related to smoking or increased levels of adducts in the lung, bronchus, larynx, bladder, cervix, and oral mucosa of smokers. In a limited number of studies, the adducts and the carcinogens responsible for them have been identified. Some studies have demonstrated higher levels of adducts in the white blood cells of smokers, while other studies indicate other sources of genotoxic agents, including diet, can contribute to the DNA damage observed in these cells.

Prabhakaran C, Kumar P, Panneerselvam N, Rajesh S, Shanmugam G. **Cytotoxic and genotoxic effects of cleistanthin B in normal and tumour cells**. Mutagenesis 1996;11(6):553-7.
Cleistanthin B, one of the toxic constituents of Cleistanthus collinus, was found to be cytotoxic to normal and tumour cells. In comparison with normal cells, tumour cells were sensitive to lower doses of toxin. The 50% growth inhibition (GI50) values for normal cell lines were from 2 x 10(-5) to 4.7 x 10(-4) M and for tumour cells the values ranged from 1.6 x 10(-6) to 4 x 10(-5) M. Short exposure (30 min) of Chinese hamster ovary (CHO) cells to cleistanthin B at 1-6 micrograms/ml resulted in extensive chromatid and isochromatid breaks and gaps. However there was no significant increase in cell death and DNA strand breaks in cells treated under the above conditions. Cleistanthin B induced micronucleus formation in cultured lymphocytes in a dose-dependent manner. CHO cells treated with high doses of cleistanthin B showed a decrease in cell viability and a concomitant increase in DNA strand-breaks. The cell death appears to be due to apoptosis since nucleosome-like ladders were observed in the treated cells when the DNA was electrophorized in agarose gels.

Reddy VR, Arumugam S. **Cytological effects of different mutagens in barley**. J Phytol Res 1995;8(1):63-6.
BIOSIS COPYRIGHT: BIOL ABS. Cytological effects due to gamma rays, EMS and their combined treatments were analysed in two barley varieties viz. K-168 and SMV-2. The gamma rays induced more aberrations than EMS and combined treatments produced even higher aberrations. The variety SMV-2 was found to be more sensitive to the mutagens than K-168.

Rupa DS, Schuler M, Eastmond DA. **Detection of hyperdiploidy and breakage affecting the 1cen-1q12 region of cultured interphase human lymphocytes treated with various genotoxic agents**. Environ Mol Mutagen 1997;29(2):161-7.

Chromosomal aberrations are associated with cancer, birth defects, and pregnancy loss. Previous studies using banding techniques have revealed that chromosomal alterations induced in human peripheral lymphocytes by many genotoxic agents occur nonrandomly throughout the genome. One of the regions prone to breakage is the centromeric heterochromatin of chromosome 1. We have developed a fluorescence in situ hybridization (FISH) procedure using tandem DNA probes to distinguish hyperdiploidy from breakage occurring in this region. Interphase nuclei exhibiting breakage or exchanges affecting the 1cen-1q12 region can readily be distinguished from nuclei hyperdiploid for this chromosome by identifying the number and location of the hybridization signals. This hybridization approach was tested using cultured human lymphocytes treated with a series of known aneuploidy-inducing agents (colchicine, diethylstilbestrol, and vincristine sulfate), several potent clastogens (ionizing radiation, mitomycin C, and etoposide), as well as sodium arsenite and hydroquinone, agents that have been reported to have relatively weak aneuploidy-inducing and clastogenic activity. Significant increases in chromosomal alterations were seen with all agents tested and the results were generally consistent with those previously seen using standard cytogenetic techniques. Treatment with colchicine, diethylstilbestrol, and vincristine sulfate resulted in high frequencies of primarily hyperdiploid nuclei, and cells exposed to radiation, mitomycin C, and etoposide exhibited elevated frequencies of breakage affecting the 1cen-1q12 region. Sodium arsenite and hydroquinone induced relatively minor but significant increases in both hyperdiploidy and breakage. These results indicate that this tandem labeling approach can be used to distinguish aneuploidy-inducing agents from those causing breakage in interphase human cells and may be a valuable procedure for monitoring human populations exposed to genotoxic agents.

Russo A, Tommasi AM, Renzi L. **Detection of minor and major satellite DNA in cytokinesis-blocked mouse splenocytes by a PRINS tandem labelling approach**. Mutagenesis 1996;11(6):547-52.
A protocol for the simultaneous visualization of minor and major satellite DNA by primed in situ DNA synthesis (PRINS) was developed in cytokinesis-blocked murine splenocytes. After individuation of optimal experimental conditions, a micronucleus (MN) test was carried out by treating splenocytes in vitro with the clastogenic agent mitomycin C and the aneugenic compound Colcemid. It was found that PRINS gives highly reproducible results, also comparable with the literature on MN results obtained by fluorescent in situ hybridization (FISH). Therefore the PRINS methodology may be proposed as a fast alternative to FISH for the characterization of induced MN.

Sage E, Lamolet B, Brulay E, Moustacchi E, Chateauneuf A, Drobetsky EA. **Mutagenic specificity of solar UV light in nucleotide excision repair-deficient rodent calls**. Proc Soc Natl Acad Sci USA 1996;93(1):176-80.
BIOSIS COPYRIGHT: BIOL ABS. To investigate the role of nucleotide excision repair (NER) in the cellular processing of carcinogenic DNA photoproducts induced by defined, environmentally relevant portions of the solar wavelength spectrum, we have determined the mutagenic specificity of simulated sunlight (3101100 nm), UVA (350-400 nm), and UVB (290-320 nm), as well as of the nonsolar model mutagen 254-nm UVC, at the adenine phosphoribosyltransferase (aprt) locus in NER-deficient (ERCC1) Chinese hamster ovary (CHO) cells. The frequency distributions of mutational classes induced by UVB and by simulated sunlight in repair-deficient CHO cells were virtually identical, each showing a marked increase in tandem CC - TT transitions relative to NER-proficient cells. A striking increase in CC - TT events was also previously documented for mutated p53 tumor-suppressor genes from nonmelanoma tumors of NER-deficient, skin cancer-prone xeroderma pigmentosum patients, compared to normal individuals. The data therefore indicate that the aprt gene in NER-deficient cultured rodent cells irradiated with artificial solar light generates the same distinctive fingerprint for sunlight mutagenesis as the p53 locus in NER-deficient humans exposed to natural sunlight in vivo. Moreover, in strong contrast to the situation for repair-competent CHO cells, where a significant role for UVA was previously noted, the mutagenic specificity of simulated sunlight in NER-deficient CHO cells and of natural sunlight in humans.

Sasaki YF, Izumiyama F, Nishidate E. [**Alkaline single cell gel electrophoresis assay with mouse multiple organs as a new in vivo genotoxicity testing system**]. Kankyo Hen'Igen Kenkyu 1997;18(3):125-36. (Jpn)
CBAC COPYRIGHT: CHEM ABS The effect of 7 model chem. mutagens on DNA was evaluated using alk. single cell gel (SCG) electrophoresis (Comet) assay in 5 mouse organs - liver, lung, kidney, spleen, and bone marrow. Mice were selected 3 and 24 h after the administration of each mutagen. Each organ was minced, suspended at a

concn. of 1 g/mL in chilled homogenizing buffer (pH 7.5) contg. 0.075M NaCl and 0.024M Na2EDTA, and homogenized gently using a Potter-type homogenizer at 500-800 rpm set in ice. Centrifuged nuclei were used for the alk. SCG assay. Two alkylating agents, MMS and ENU; a crosslinking agent, MMC; an arom. amine, 2AAF; a polycyclic arom. hydrocarbon, B[alpha]P; and an inorg. chem., KBrO3, were genotoxic. Colchicine, on the other hand, produced neg. responses. These results demonstrated that the alk. SCG assay detects the mutagenic effects of chems. in a manner predicted by their mode of action. The genotoxic effect of 4 liver-targeting carcinogens was evaluated using alk. SCG assay. The selected carcinogens produced neg. responses in the micronucleus test. Auramine, p-dimethylaminoazobenzene, phenobarbital sodium, and styrene 7,8-oxide induced DNA damage, mainly in the liver. Thus, it may be possible to use the alk. SCG assay to detect the genotoxicity of chems. in vivo in their target organs. The in vivo alk. SCG assay with this modification was shown to be applicable to rats, suggesting that this assay could be combined with general toxicity tests.

Sasaki YF, Tsuda S, Izumiyama F, Nishidate E. **Detection of chemically induced DNA lesions in multiple mouse organs (liver, lung, spleen, kidney, and bone marrow) using the alkaline single cell gel electrophoresis (Comet) assay**. Mutat Res1997; 388(1):33-44.
The effect of 2 model chemical mutagens on DNA was evaluated with the alkaline single cell gel electrophoresis (SCG) (Comet) assay in 5 mouse organs--liver, lung, kidney, spleen and bone marrow. Mice were sacrificed 3 and 24 h after the administration of the direct mutagen ethyl nitrosourea (ENU) or the liver-targeting promutagen p-dimethylaminoazobenzene (DAB). Each organ was minced, suspended at a concentration of 1 g/ml in chilled homogenizing buffer (pH 7.5) containing 0.075 M NaCl and 0.024 M Na2EDTA, homogenized gently using a Potter-type homogenizer at 500-800 rpm set in ice, and then centrifuged nuclei were used for the alkaline SCG assay. ENU induced DNA damage in cells all of the organs studied DAB, on the other hand, produced a positive response in the liver only. We suggest that it may be possible to use the alkaline SCG assay using a homogenization technique to detect the genotoxicity of chemicals in vivo in their target organs.

Savva D, Castellani S. **Environmental contamination. a new molecular technique to complement cytogenetic analysis**. Arch Zootec 1996;45(170-171):175-81.
CBAC COPYRIGHT: CHEM ABS A review and discussion with 24 refs. The effects of environmental pollutants on organisms may be monitored in a no. of ways and at different levels. Exposure to genotoxic chems. results in the formation of covalently bound adducts between the genotoxin and the DNA and these may cause mutations and cytogenetic changes. The primary effects on DNA (i.e. adduct formation) may be monitored using 32P-postlabeling, ELISA or HPLC. Secondary effects on DNA (cytogenetic damage, mutation) may be monitored using a no. of biomarker assays capable of detecting DNA strand breaks (e.g. by the alk. unwinding assay or the Comet assay), unscheduled DNA synthesis, micronuclei, chromosome aberrations, sister chromatid exchanges and phenotypic and genotypic changes due to mutation. The sensitivity and specificity of these assays is variable. Recent developments in mol. biol. such as DNA fingerprinting and gene amplification by the polymerase chain reaction (PCR) offer new possibilities for detecting DNA damage. The authors examd. whether an alternative biomarker assay using DNA fingerprinting by arbitrarily primed PCR (AP-PCR) can show differences in the DNA fingerprints of individual animals exposed to benzo(a)pyrene in the lab. and of animals from control and from polluted areas. The results indicate that DNA fingerprinting by AP-PCR offers a useful alternative biomarker assay for the detection of the genotoxic effects of environmental pollutants.

Schiestl RH, Aubrecht J, Khogali F, Carls N. **Carcinogens induce reversion of the mouse pink-eyed unstable mutation**. Proc Natl Acad Sci USA 1997;94(9):4576-81.
Deletions and other genome rearrangements are associated with carcinogenesis and inheritable diseases. The pink-eyed unstable (pun) mutation in the mouse is caused by duplication of a 70-kb internal fragment of the p gene. Spontaneous reversion events in homozygous pun/pun mice occur through deletion of a duplicated sequence. Reversion events in premelanocytes in the mouse embryo detected as black spots on the gray fur of the offspring were inducible by the carcinogen x-rays, ethyl methanesulfonate, methyl methanesulfonate, ethyl nitrosourea, benzo [a]pyrene, trichloroethylene, benzene, and sodium arsenate. The latter three carcinogens are not detectable with several in vitro or in vivo mutagenesis assays. We studied the molecular mechanism of the carcinogen-induced

reversion events by cDNA analysis using reverse transcriptase-PCR method and identified the induced reversion events as deletions. DNA deletion assays may be sensitive indicators for carcinogen exposure.

Sehlmeyer U, Rohwedel J, Wobus AM. **Primordial germ cell-derived embryonic germ cells of the mouse: in vitro model for cytotoxicity studies with chemical mutagens**. Toxicol In Vitro 1996;10(6):755-63.
BIOSIS COPYRIGHT: BIOL ABS. Different screening methods to detect the toxic effects of xenobiotics using cells from vertebrates and invertebrates in cytotoxicity and viability assays have been developed, but up to now appropriate in vitro methods with mammalian germ cells have not been available. In the present study the primordial germ (PG) cell-derived permanent embryonic germ (EG) cell line EG-1 was used as in vitro model in toxicity studies with chemical mutagens. EG-1 cells and embryonic stem cells of line D3 were comparatively investigated for their cell survival in response to N-ethyl-N-nitrosourea (ENU), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and mitomycin C (MMC) and the results compared with those obtained for undifferentiated embryonic carcinoma cells of line P19 and differentiated epithelioid EPI-7 cells. As a prerequisite for in vitro toxicity and viability studies the cultivation conditions for EG-1 and D3 cells in the absence of a feeder layer were improved by a conditioned medium. increasing the plating efficiency from 0.08% to 17.5% and from 21.1% to 25.1% for EG-1 and D3 cells, respectively. The resulting mean generation time (MGT) of 16.9 hr for EG-1 cells was identical to the generation time of PG cells in vivo, and was not significantly different from the MGT of D3 (15.6 hr) and EPI-7 (13.7 hr) cells, but significantly longer than the MGT of P19 cells (9.3 hr). Calculations of the concentrations resulting in vitro in a 50% decrease in cell survival demonstrated that EG-1 cells were more sensitive to the toxic effects of ENU, MNNG and MMC than D3 and P19 cells and, with the exception of MNNG, also more sensitive than EPI-7 cells. It is proposed that EG cells are used as a model system to screen for toxic effects of teratogenic and embryotoxic chemical agents in vitro.

Sengupta RK, Ghosh P. **Genotoxic effects of lead nitrate on pea plant**. J Phytol Res 1995;8(2):107-14.
BIOSIS COPYRIGHT: BIOL ABS. Cytogenetical effects of lead nitrate were investigated through seed soak treatment of Pisum sativum L. Effects were steadily increased with the increase in dose and duration. The probable mechanism responsible for producing chromosomal anomalies and phenotypic alterations have been discussed in detail. Effects were diluted in successive generation but it is difficult to interprete that it is entirely safe from genotoxic point. The appearance of chromosome breakage, cytomiotic cells and anaphasic bridges that chemical can affect genetic recombination which may lead to loss of important factors or gain undesirable characters. It can be concluded that lead nitrate is a cytotoxic chemical mutagen reduces plant growth as well as yield.

Shelby MD, Tindall KR. **Mammalian germ cell mutagenicity of ENU, IPMS and MMS, chemicals selected for a transgenic mouse collaborative study**. Mutat Res 1997;388(2-3):99-109.
A collaborative study to systematically assess transgenic mouse mutation assays as screens for germ cell mutagens has been conducted. Three male mouse germ cell mutagens (ENU, iPMS and MMS) were selected for testing. This paper provides a brief review of the effects reported for those 3 chemicals in the most commonly used non-transgenic germ cell mutagenicity assays, namely the dominant lethal, heritable translocation, and specific locus tests. Additionally, information on the DNA reactivity and the molecular nature of mutations induced by these chemicals is summarized.

Shimizu H, Yagi R, Kimura Y, Makino K, Terato H, Ohyama Y, Ide H. **Replication bypass and mutagenic effect of alpha-deoxyadenosine site-specifically incorporated into single-stranded vectors**. Nucleic Acids Res 1997:25(3):597-603.
alpha-2'-Deoxyadenosine (alpha) is a major adenine lesion produced by gamma-ray irradiation of DNA under anoxic conditions. In this study, single-stranded recombinant M13 vectors containing alpha were constructed and transfected into Escherichia coli to assess lethal and mutagenic effects of this lesion. The data for alpha were further compared with those obtained with M13 vectors containing normal A or a model abasic site (F) at the same site. The transfection assay revealed that alpha constituted a moderate block to DNA replication. The in vivo replication capacity to pass through alpha was approximately 20% relative to normal A, but 20-fold higher than that

of F constituting an almost absolute replication block. Similar data were obtained by in vitro replication of oligonucleotide templates containing alpha or F by E.coli DNA polymerase I. The mutagenic consequence of replicating M13 DNA containing alpha was analyzed by direct DNA sequencing of progeny phage. Mutagenesis was totally targeted at the site of alpha introduced into the vector. Mutation was exclusively a single nucleotide deletion and no base substitutions were detected. The deletion frequency associated alpha was dependent on the 3'-nearest neighbor base: with the 3'-nearest neighbor base T mutation (deletion) frequency was 26%, whereas 1% with the 3'-nearest neighbor base G. A possible mechanism of the single nucleotide deletion associated with alpha is discussed on the basis of the misinsertion-strand slippage model.

Slamenova D, Gabelova A, Ruzekova L, Chalupa I, Horvathova E, Farkasova T, Bozsakyova E, Stetina R. **Detection of MNNG-induced DNA lesions in mammalian cells; validation of comet assay against DNA unwinding technique, alkaline elution of DNA and chromosomal aberrations**. Mutat Res 1997;383(3):243-52.
CBAC COPYRIGHT: CHEM ABS Human cells (VH10 or Hep G2) and hamster cells V79 were exposed to different concns. of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and the level of DNA lesions was evaluated by the DNA unwinding technique, alk. elution of DNA and the comet assay. All three methods were able to detect the effects of MNNG but with a clear difference in sensitivity. At low concns. of MNNG the most sensitive method appeared to be the comet assay. After the short-term treatment the comet assay was able to detect the lesions induced by MNNG at approx. 0.1 mug/mL, alk. elution of DNA at 1 mug/mL and DNA unwinding at 1-2 mug/mL. MNNG treated VH10 cells, human lymphocytes and V79 cells were also tested cytogenetically, confirming that MNNG induced chromosomal aberrations at concns. >1 mug/mL in VH10 cells (short-term treatment); >0.2 mug/mL in V79 cells (long-term treatment) and >8 mug/mL in human lymphocytes (long-term treatment). In some expts. we tried to increase the level of MNNG-induced DNA breaks with help of DNA repair inhibitors cytosine arabinoside (Ara C) and hydroxyurea (HU) which were applied either after or during MNNG treatment. Our results showed that the level of MNNG-induced lesions was increased by simultaneous treatment of cells with MNNG and Ara C and HU; 2. times.10-5 M Ara C and 2.times.10-3 M HU were as effective as 10-times higher concns. of inhibitors. Ara C and HU increased the level of MNNG-induced DNA breaks mainly in combination with lower concns. of MNNG (<2 MUG/ML). Rejoining of DNA breaks was obsd. in human cells VH10 and HEP G2 as well as in chinese hamster cells V79 damaged by both lower and higher MNNG-concns. All methods showed that NNG-induced DNA breaks had been gradually rejoined.

Speit G, Hartmann A. [**Detection of environmental genotoxic agents with the comet assay**]. Proj Angew Oekol 1996;20:114 Pp. (Ger)
CBAC COPYRIGHT: CHEM ABS The comet assay was applied to detect DNA damage caused by direct and indirect mutagens in human cells, in vitro. DNA strand breaking and formation of alkali-labile sites were detected by the assay, but damage by crosslinking subtances was less evidenced. Studies with cytotoxic substances led to neg. results, thus indicating that the comet assay is specific for genotoxic effects. The effect of DNA repair on the comet assay was complex. In-vivo and population studies were also carried out.

Spitz MR, Wu X, Jiang H, Hsu TC. **Mutagen sensitivity as a marker of cancer susceptibility**. J Cell Biochem Suppl 1996;25:80-4.
Modulation of environmental exposures by host genetic factors may explain interindividual variation in susceptibility to carcinogenesis. One determinant of susceptibility is mutagen sensitivity measured by the frequency of bleomycin-induced breaks in an in vitro lymphocyte assay. Mutagen sensitivity is a significant predictor of aerodigestive tract cancer risk. In this case-control study of lung-cancer susceptibility markers, 54% of 132 lung-cancer cases had mutagen-sensitivity scores greater than or equal to 1 break/cell, compared with only 22% of 232 controls. The mean breaks/cell value (+/-SE) for the 88 African-American cases was 1.11 (+/-0.60), compared with 0.82 (+/-0.49) for the 121 controls (P < 0.001). for the 44 Mexican-American cases and 111 controls, the comparable values were 1.11 (+/-0.52) and 0.76 (+/-0.38), respectively. The overall odds ratio (or) for mutagen sensitivity (dichotomized at > or = 1 break/cell), after adjusting for ethnicity and smoking status, was 3.62 (95% confidence limits [CL] = 2.2, 5.9). For current smokers the adjusted risk associated with mutagen sensitivity was 2.52 (1.2, 5.3). For former smokers, the comparable OR (95% CL) was 6.19 (2.7, 14.1). The risk estimate for those under 61 years of age was 4.85 (2.3,

10.4), compared with 2.85 (1.5, 5.6) for older subjects. The risk also appeared to be higher for lighter smokers (< 20 cigarettes daily) than heavier smokers (ORS = 5.72 AND 3.20, respectively). The ethnicity-adjusted ORS by quartile of breaks/cell were 1.0, 1.40, 2.46, and 4.80; the trend test was significant at P < 0.001. The joint effects of mutagen sensitivity and former smoking, current smoking , or heavy smoking were greater than additive, although the interaction terms were not statistically significant in the logistic model. Mutagen sensitivity may therefore be a useful member of a panel of susceptibility markers for defining high-risk subgroups for chemoprevention trials.

Sram RJ. **Future research directions to characterize environmental mutagens in highly polluted area**. Environ Health Perspect 1996;104(Suppl 3):603-7.
Population monitoring using methods of molecular epidemiology combined with reliable data on exposure is an extremely powerful approach to determine the effect of mutagens on human populations. Although human blood and urine have traditionally been used for biomonitoring, an increase in the use of placental and buccal smear samples should be expected. As biomarkers of exposure, DNA strand breaks and hemoglobin and albumin adducts seem to be most sensitive. As biomarkers of response, cytogenetic analysis determining chromosome aberrations or micronuclei has been widely used Additional information can be obtained by using the chromosome painting technique and by determining gene mutations at the hprt locus: however, epidemiological studies exhibiting a relationship between these biomarkers and environmental pollution are still lacking. The use of sperm to analyze the effect of environmental mutagens in germ cells (e.g, sperm morphology and sperm aneuploidy) should be encouraged. The determination of susceptibility by analyzing genetic polymorphism, which is responsible for individual differences in the biotransformation of mutagens and carcinogens, will gain importance for risk assessment. Future research should include validating molecular methods, studying adaptive response to chemical carcinogens, and studying the modulatory effect of antioxidants, as well as the effect of carcinogens on immunity.

Tafazoli M, Kirsch-Volders M. **In vitro mutagenicity and genotoxicity study of 1,2-dichloroethylene, 1,1,2-trichloroethane, 1,3-dichloropropane, 1,2,3-trichloropropane and 1,1,3-trichloropropene, using the micronucleus test and the alkaline single cell gel electrophoresis technique (comet assay) in human lymphocytes**. Mutat Res 1996;371(3-4):185-202.
BIOSIS COPYRIGHT: BIOL ABS. The main objective of this study was to compare the cytotoxic genotoxic and mutagenic activity of a number of chlorinated aliphatic hydrocarbons, which are widely used as chemical intermediates, solvents, degreasing agents etc. in industry, and to establish the structure-toxicity relationship of the chemicals by using the most adequate determinants in estimating their toxicity. The mutagenicity and cytotoxicity of some of the candidate chemicals, namely 1,2-dichloroethylene, 1,1,2-trichloroethane, 1,3-dichloropropane, 1,2,3-trichloropropane and 1,1,3-trichloropropene were evaluated in an in vitro micronucleus assay. The cytokinesis-block methodology was applied on human lymphocytes in the presence or absence of an external metabolic activation system (S9-mix). In the micronucleus assay, all test substances, except 1,2,3-trichloropropane with and without S9-mix and 1,1,2-trichloroethane without S9-mix in the repeated experiment, exhibited a low but statistically significant mutagenic activity, compared to the concurrent control. However, none of the five chemicals was able to induce a clear and reproducible linear dose-dependent increase in micronucleus frequencies in this assay. Generally, mutagenic activity of the chemicals was found in the absence of severe cytotoxicity and/or cell cycle delay. The DNA breakage capacity and the cytotoxicity of these chemicals were also assessed in the alkaline single cell gel (SCG) electrophoresis test (comet assay) with and without S9-mix in isolated human lymphocytes. All chemical compounds induced DNA breakage, in the presence or absence of the metabolic activation system, at the doses tested. The data showed that the DNA reactivity of the chemicals increased with increasing degree of halogenation. The results of the present work suggested that the comet assay might be a more suitable and sensitive screening method than the micronucleus test for this particular class of compound. However, both assays do detect different endpoints.

Tice RR, Stack HF, Waters MD. **Human exposures to mutagens--an analysis using the genetic activity profile database**. Environ Health Perspect 1996;104(3):585-9.
The Genetic Activity Profile (GAP) database was used to identify and compare agents showing genotoxic activity in humans. The database revealed several substances for which both human and rodent cytogenetic data existed.

Based on the ratio of the lowest effective doses (LEDs) in rodents versus human studies, humans appear to be at least 10 times more sensitive than rodents to the majority of the genotoxic substances examined. Several caveats are discussed which may be responsible, in part, for the apparent differences in sensitivity. Some of these differences could be due to variations in the test protocols or they may, in fact, reflect real differences between human and rodent cells. However, in contrast to the in vivo comparison, the LEDs for human data from in vitro studies were not uniformly lower than for comparable studies in rodents. The in vitro comparison suggests that the apparent differences in human versus rodent cell sensitivity seen in vivo must be viewed with a degree of caution. Nevertheless, the overall GAPs for these agents, and particularly the human in vivo data, underscore the concern for adequate protection of humans exposed to these environmental mutagens.

Ticho BS, Stainier DY, Fishman MC, Breitbart RE. **Three zebrafish MEF2 genes delineate somitic and cardiac muscle development in wild-type and mutant embryos**. Mech Dev 1996;59(2):205-18.
The zebrafish is an important experimental system for vertebrate embryology, and is well suited to the molecular analysis of muscle development. Transcription factors, such as the MEF2s, regulate skeletal and cardiac muscle-specific genes during development. We report the identification of three zebrafish MEF2 genes which, like their mammalian counterparts, encode factors that function as DNA-binding transcriptional activators of muscle specific promoters. The pattern of MEF2 expression in zebrafish defines discrete cell populations in the developing somites and heart and has mechanistic implications for developmental regulation of the MEF2 genes, when compared with other species. Alteration of MEF2 expression in two mutants affecting somitogenesis provides insight into the control of muscle formation in the embryo.

Trosko JE. **Challenge to the simple paradigm that 'carcinogens' are 'mutagens' and to the in vitro and in vivo assays used to test the paradigm**. Mutat Res 1997;373(2):245-9.

Van Der Lelie D, Regniers L, Borremans B, Provoost A, Verschaeve L. **The VITOTOX test, an SOS bioluminescence Salmonella typhimurium test to measure genotoxicity kinetics**. Mutat Res 1997;389(2-3):279-90.
A new test to detect genotoxicity, that we refer to as the VITOTOX test, was developed. Four gene fusions that are based on the Escherichia coli recN promoter were constructed and evaluated for their SOS response-dependent induction. The wild-type recN promoter, a derivative mutated in the second LexA binding site, a derivative with a mutated -35 region, and a derivative from which both the second LexA binding site and the -35 region were mutated, were cloned upstream of the promoterless Vibrio fischeri luxCDABE operon of pMOL877, in such a way that lux became under transcriptional control of the recN promoter derivatives. The inducibility by the SOS response of the promoter constructs was tested in both E. coli and in the Ames test Salmonella typhimurium strains TA98, TA100 and TA104. In all strains, the highest sensitivity and induction was observed with the plasmids pMOL1067 and pMOL1068, that contain the lux operon under control of the recN promoter mutated in the second LexA binding site, or a recN promoter with a mutated -35 region, respectively. Therefore, strains containing pMOL1067 or pMOL1068 were further used for genotoxicity testing. With the VITOTOX test, genotoxicity was detected within 1-4 h. The VITOTOX test is very sensitive: for most products tested, the minimal detectable concentration (MDC) values were considerably lower (5 to > 100 times) than those described for the Ames test and the SOS chromotest. A good correlation was observed with the results from the Ames tests, but certain PAHs that are not mutagenic in the Ames test were genotoxic in the VITOTOX test. With the VITOTOX strains, the kinetics of SOS induction can be determined. This feature made it possible to distinguish between compounds in mixtures of genotoxic products so long as they had different induction kinetics.

Vismara C, Garavaglia A. **4-Chloro-2-methylphenoxyacetic acid containing compounds. Genotoxicity evaluation by Mutatox assay and comparison with acute (Microtox) and embryo (FETAX) toxicities**. Bull Environ Contam Toxicol 1997;58(4):582-8.
CBAC COPYRIGHT: CHEM ABS Genotoxicity of title herbicide MCPA, MCPA Na salt, com. formulation Erbitox E30, tech. grade MCPA Na salt, and 2 of its intermediates phenol and chloro-cresol were evaluated using Mutatox,

and the Mutatox data were compared with the other 2 title assays. Erbitox E30 was the least mutagenic of the tested compds. In case of this compd. only 28% of the photoactive compd., with a purity of 87% is present while the remaining is made of an unknown mixt. of additives. The difference in the light unit value (the light exited by the treated bacteria to the av. blank value) between MCPA Na and its tech. grade prepn. was attributed to the presence of phenol and chloro-cresol in the tech. grade. All the tested compds., including phenol and chloro-cresol, did not show any signs of mutagenic effects after the treatment with exogenous metabolic activation system (S9) as mammalian liver detoxication nullifies the mutagenic activity of these compds.

Vismara C, Rossetti C, Bolzacchini E, Orlandi M, Luperini A, Bernardini G. **Toxicity evaluation of 4-chloro-2-methylphenoxyacetic acid by Microtox and comparison with FETAX**. Bull Environ Contam Toxicol 1996;56 (1):85-9.

Visvardis E, Tassiou AM, Piperakis SM. **Study of DNA damage induction and repair capacity of fresh and cryopreserved lymphocytes exposed to H2O2 and gamma-irradiation with the alkaline comet assay**. Mutat Res 1997;383(1):71-80.
CBAC COPYRIGHT: CHEM ABS DNA lymphocyte hydrogen peroxide gamma ray;DNA damage DNA damage induction and repair in human lymphocytes exposed to H2O2 and gamma-rays studied by alk. comet assay;DNA repair DNA damage induction and repair in human lymphocytes exposed to H2O2 and gamma-rays studied by alk. comet assay;Gamma ray DNA damage induction and repair in human lymphocytes exposed to H2O2 and gamma-rays studied by alk. comet assay;Lymphocyte DNA damage induction and repair in human lymphocytes exposed to H2O2 and gamma-rays studied by alk. comet assay.

Wei Q, Spitz MR, Gu J, Cheng L, Xu X, Strom SS, Kripke ML, Hsu TC. **DNA repair capacity correlates with mutagen sensitivity in lymphoblastoid cell lines**. Cancer Epidemiol Biomarkers Prev 1996;5(3):199-204.
This study describes a correlation between cellular DNA repair capacity and the frequency of mutagen-induced in vitro chromosomal breaks in selected lymphoblastoid cell lines. Two assays, host cell reactivation (HCR) assay for measuring cellular DNA repair capacity and in vitro mutagen sensitivity assay, have recently been shown to be useful biomarkers for such susceptibility. Increased in vitro mutagen sensitivity, measured by the number of induced chromatid breaks, has been postulated to reflect decreased capacity of DNA repair, as measured by the HCR assay. However, these two assays have not been examined in parallel to test this hypothesis. In this study, we performed both assays in 16 established lymphoblastoid cell lines derived from patients with xeroderma pigmentosum (n = 3), ataxia telangiectasia (n = 2), head and neck cancer (n = 3), and melanoma (n = 2), and from normal human subjects (n = 6) using UV light, 4-nitroquinoline-1-oxide (4-NQO; an UV-mimetic agent), and gamma-irradiation as the test agents. The measurements from the HCR assay correlated significantly with the frequency of chromatid breaks induced by either UV irradiation ($r = -0.69$; $P < 0.01$) OR 4-NQO ($R = -0.70$; $P < 0.01$). Although published data suggest that damage induced by UV and 4-NQO maybe repaired by different pathways, the two agents induced similar frequencies of chormotid breaks ($R = 0.68$; $P < 0.01$) in the tested cell lines.

Winegar RA, Carr G, Mirsalis JC. **Analysis of the mutagenic potential of ENU and MMS in germ cells of male C57BL/6 lacI transgenic mice**. Mutat Res 1997;388(2-3):175-8.
Mutant frequencies in male germ cells were determined in mice 3 days after exposure to saline, methylmethane sulfonate (MMS), or ethylnitrosourea (ENU). DNA was isolated from seminiferous tubules by a modified version of the drop dialysis method. A 5-fold increase in mutant frequency was observed in mice treated with ENU. No statistically significant increase was observed in mice treated with MMS.

Wojewodzka M, Kruszewski M, Szumiel I. **Effect of signal transduction inhibition in adapted lymphocytes: micronuclei frequency and DNA repair**. Int J Radiat Biol 1997;71(3):245-52.
Irradiation of human lymphocytes (1 cGy X rays, 37 degrees C) or their treatment with 10 microM hydrogen peroxide (30 min at 37 degrees C) evoked a ca 30% decrease in the frequency of micronuclei upon subsequent X-irradiation (1.5 Gy). The response was reflected in a lower micronuclei frequency, but no change in DNA repair rate

was observed as measured by the comet assay, directly after the challenge dose. Treatment of lymphocytes with staurosporine, an inhibitor of protein kinases, or with TMB-8, a calcium antagonist, carried out in parallel with the adaptive dose prevented the development of the adaptive response measured as micronuclei frequency. In lymphocytes that were staurosporine- or TMB-8-treated and irradiated under adaptive conditions showed that the rate of DNA repair was not changed. We conclude that treatment with agents that interfere with the transduction of the signal triggered by the low dose prevents the development of the adaptive response induced by X rays or hydrogen peroxide. Lower chromosome damage revealed by the cytokinesis block-micronuclei test in the adapted lymphocytes is unrelated to DNA repair rate as measured by comet assay.

Zakharenko LP, Zakharov IK, Vasyunina EA, Karamysheva TV, Danilenko AM, Nikiforov AA. **Determination of genotoxicity of fullerene C60 and fullerol by somatic mutation and recombination test in Drosophila melanogaster and SOS chromotest**. Russ J Genet 1997;33(3):327-30.
CBAC COPYRIGHT: CHEM ABS Genotoxicity of fullerene C60 was detd. in a prokaryotic in vitro test and in an eukaryotic in vivo system. The SOS chromotest of fullerene C60 in the Escherichia coli strain PQ37 revealed no genotoxicity either with or without activation of the rat liver homogenate. To perform the somatic mutation and recombination genotoxicity test (SMART) on somatic wing cells, Drosophila melanogaster larvae were grown on a std. medium with or without fullerene dope. No statistically significant differences were obsd. at the same fullerene concns. in the SOS chromotest (0.45 mug/mL). Only at the highest possible fullerene concn. of 2.24 mug per 1 mL medium, a slight genotoxic effect was obsd. in wing cells. Fullerol demonstrates no mutagenic effect at a concn. of 2.46 mg/mL.

Zeiger E, Ashby J, Bakale G, Enslein K, Klopman G, Rosenkranz HS. **Prediction of Salmonella mutagenicity**. Mutagenesis 1996;11(5):471-84.
The ability of a number of prediction systems was examined to determine how well they could predict Salmonella mutagenicity. The prediction systems included two computer-based systems (CASE and TOPKAT), the measurement of a physiochemical parameter (ke) and the use of structural alerts by an expert chemist. The computer-based systems operators and the chemist were supplied with the structures of 100 chemicals that had been tested for mutagenicity in the Salmonella test; the actual chemicals were needed for the physiochemical measurement. None of the participants was provided with the chemical names or Salmonella test results prior to submitting their predictions. The three systems that predicted the mutagenicity from the structure of the chemicals produced equivalent results (71-76% concordance with the Salmonella results); the physiochemical system produced a lower (60-61%) concordance.

HEPATIC AND RENAL TOXICITY

Bisgaard HC, Nagy P, Santoni-Rugiu E, Thorgeirsson SS. **Proliferation, apoptosis, and induction of hepatic transcription factors are characteristics of the early response of biliary epithelial (oval) cells to chemical carcinogens**. Hepatology 1996;23(1):62-70.
BIOSIS COPYRIGHT: BIOL ABS. In this study, we used (3H)thymidine labeling of newly synthesized DNA to examine the earliest effects of 2-acetylaminofluorene (2-AAF) on the mitotic activation of cells in the adult rat liver, and in situ hybridization analysis to study the expression of three transcription factors (HNF1beta, HNF3gamma, and HNF4), and two of the genes (alpha-fetoprotein (AFP) and albumin) regulated by these factors. A low dose of 2-AAF (and its analogs, 2-AAF (2aminofluorene) and N-OH-2-AAF) elicited a mitogenic response in ductal cells and nondescript periductular cells within 24 hours after administration. The compounds also induced the expression of HNF1beta, HNF3gamma, AFP, and albumin in ductal structures but had no detectable effect of HNF4 expression. In contrast, initiation of bile duct proliferation by ligation of the common bile duct had no effect on the expression of these genes in ductal cells. In addition to inducing a mitogenic response, 2-AAF resulted in increased numbers of apoptotic cells in the portal areas, a process that contributed to overall retention of liver morphology. Our results

demonstrate that 2-AAF and some of its analogs can elicit a specific mitogenic response and induce expression of the establishment transcription factors, HNF1beta and HNF3gamma, in ductal cells. Our data provide further support of a precursor-product relationship between stem-like cells located in ductal structures, oval cells, and hepatocytes.

Decicco LA, Kong J, Ringer DP. **Carcinogen-induced alteration in liver epidermal growth factor receptor distribution during the promotion stage of hepatocarcinogenesis in rat**. Cancer Lett 1997;111(1-2):149-56.

Di Stasi SM, Vespasiani G, Giannantoni A, Massoud R, Dolci S, Micali F. **Electromotive delivery of mitomycin C into human bladder wall**. Cancer Res 1997;57(5):875-80.
CBAC COPYRIGHT: CHEM ABS The aim of this investigation was to establish an appropriate tissue pharmacokinetic model to compare concns. of mitomycin C (MMC) in the human bladder wall after either passive delivery or electromotive administration (EMDA) and to evaluate the effects of EMDA on tissue morphol. and MMC structure. Tissue sections of human bladder were inserted into 2 chamber cells, with the urothelium exposed to the donor compartment contg. MMC (10 mg in 100 mL of 0.24% NaCl soln.) and an anode, and with the serosa exposed to the receptor compartment contg. 100 mL of 0.9% NaCl soln. and a cathode. The tissue was exposed for 15 min, either without (control) or with a current of 5 mA; tissue MMC content was assessed by HPLC. Tissue viability and morphol. and MMC stability were assessed by the trypan blue exclusion test, tissue pH, histol. anal., and mass spectrometry. MMC concns. were increased, and the variability in drug uptake was reduced, in all samples exposed to elec. current. Tissues were viable and undamaged histol., and no MMC structural modification was obsd. Thus, EMDA enhances administration of MMC into viable bladder wall tissue and reduces the variability of drug delivery rates.

Katayama T, Cheng CC, Egashira Y, Ohta T, Sanada H. **Effect of dietary L-glutamine on the hepatotoxic action of D-galactosamine in rats**. Biosci Biotechnol Biochem 1996;60(9):1425-9.
The protective effect of dietary L-glutamine against the hepatotoxic action of D-galactosamine (GaIN) was investigated by model experiments with rats. Rats fed with 20% casein diets containing 10% free amino acids were injected with GaIN, and the serum aspartate aminotransferase, alanine aminotransferase and lactate dehydrogenase activities and the hepatic glycogen content were assayed 20 hours after the injection. These enzyme activities in the group fed with 10% L-glutamine diet for 8 days were lower than those in the groups fed with the control, 10% L-glutamic acid and 10% L-alanine diets for 8 days. The more prolonged the feeding period with the 10% L-glutamine diet was, the more the serum activity levels of such enzymes were decreased. Although neomycin also lowered these enzyme activities, its simultaneous ingestion with neomycin did not show any additive or synergistic effect. The hepatic glycogen content in the 10% glutamine group still remained high after the GaIN treatment. It is therefore assumed that the effectiveness of glutamine intake would have been mediated by glycogen metabolism rather than by uridine metabolism.

Leist M, Gantner F, Naumann H, Bluethmann H, Vogt K, Brigelius-Flohe R, Nicotera P, Volk HD, Wendel A. **Tumor necrosis factor-induced apoptosis during the poisoning of mice with hepatotoxins**. Gastroenterology 1997;112(3):923-34.
BACKGROUND & AIMS: Treatment with tumor necrosis factor (TNF) induces murine hepatocyte apoptosis in vitro and in vivo when sensitizing concentrations of toxins are present. The aim of this study was to investigate whether endogenously formed TNF contributes to liver failure caused by hepatotoxins. METHODS: The extent of liver damage, induced by alpha-amanitin or actinomycin D (ActD), was examined under various experimental conditions, preventing the action of TNF on hepatocytes. RESULTS: TNF induced apoptosis of murine hepatocytes or human hepatoma cells in the presence of alpha-amanitin or ActD. TNF and alpha-amanitin induced such hepatotoxicity also in vivo in a synergistic way. After in vivo administration of high doses of ActD or alpha-amanitin alone, hepatic TNF-messenger RNA was increased and hepatocytes underwent apoptosis. A neutralizing antiserum against TNF-alpha prevented the liver injury. Hepatotoxicity of ActD or alpha-amanitin also was prevented by pretreatment of mice with low doses of the tolerizing cytokine interleukin 1. Mice deficient for the 55-kilodalton TNF receptor were

protected from ActD- or alpha-amanitin-induced toxicity. Endotoxin-unresponsive C3H/HeJ mice also had liver failure after ActD treatment, and this damage was prevented by treatment with anti-TNF antiserum. CONCLUSIONS: Hepatotoxins such as alpha-amanitin may induce liver failure by an indirect mechanism involving sensitization of parenchymal cells toward endogenously produced TNF.

Maxuitenko YY, Curphey TJ, Kensler TW, Roebuck BD. **Protection against aflatoxin B1-induced hepatic toxicity as short-term screen of cancer chemopreventive dithiolethiones**. Fundam Appl Toxicol 1996;32 (2):250-9.
Dithiolethiones are an important class of cancer chemopreventive agents. More than 50 new dithiolethione analogs were synthesized for structure-activity studies. Using selected dithiolethiones, studies were designed to measure protection against the hepatotoxicity of aflatoxin B1 (AFB1) and relate it to the protection against carcinogenicity. Young male F344 rats were pretreated with 0.1 or 0.3 mmol dithiolethiones/kg body wt and challenged with toxic doses of AFB1 (50 micrograms/100 g rat/day) on 2 successive days. One day later, the protection from hepatotoxicity was assessed by measuring serum hepatic enzymes, hepatic necrosis, and degree of bile duct cell proliferation. The ability of these dithiolethiones to prevent AFB1-induced tumorigenicity was assessed by quantifying the hepatic burden of putative preneoplastic lesions [placental glutathione S-transferase (GST-P)-positive foci]. Significant correlations ($p < 0.01$) were observed between these toxicological indices and GST-P focal burden (Alanine Aminotransferase, $R = 0.943$; Sorbitol dehydrogenase, $R = 0.897$; Histological index, $R = 0.893$; bile duct cell proliferation, $R = 0.933$). These results imply that inhibition of hepatotoxicity affords protection against hepatocarcinogenicity. The extent of protection from acute hepatotoxicity offeres a simple, short-term biological endpoint to screen dithiolethiones and related compounds for their chemopreventive properties.

Obata T, Yamanaka Y. **Monoamine oxidase released into plasma treated with the hepatotoxin allyl formate**. Res Commun Mol Pathol Pharmacol 1996;92(3):365-8.
Monoamine oxidase (MAO) released from the rat liver into plasma and the activity levels of lipid peroxide (LPO) and superoxide dismutase (SOD) in liver tissue were investigated after pretreatment of rats with the perilobular hepatotoxin allyl formate (AF). When 3H-pargyline was given to AF-pretreated rats, the levels of 3H-pargyline labelled MAO in rat plasma were increased to 38% ($p < 0.01$ vs control), but the radioactivity was decreased to 35% ($P < 0.05$ vs control). The molecular weight of MAO subunits in plasma was similar to that of MAO subunits in liver (about 60,000) as determined by SDS electrophoresis. Liver tissue LPO levels in these rats were increased, whereas SOD activitity was decreased. These results suggest that MAO in liver mitochondria was released into plasma as a consequence of membrane disorder.

Powell CJ, Secretan MB. **Induction and reversibility of genotoxic and nongenotoxic carcinogen-induced altered hepatocyte foci in rats**. Toxicol Path 1995;23(6):757-8.

Roberts RA. **Non-genotoxic hepatocarcinogenesis suppression of apoptosis by peroxisome prolfierators**. Ann N Y Acad Sci 1996:804:588-611.

Yoo SY, Kim KW, Lee HJ, Choi YC. **In vitro regeneration of carcinogen thioacetamide treated rat hepatocytes**. Korean J Pharm 1997;32(3):399-406.
BIOSIS COPYRIGHT: BIOL ABS. Thioacetamide is a non-genotoxic carcinogen, a protein modifying agent. It causes nucleolar hypertrophy in short term treatment. In the present work, thioacetamide treated hepatocytes were observed in vivo and in vitro conditions. After 7 day treatment of rat liver with thioacetamide, the hepatocyte nucleoli were enlarged and their signalling molecules such as B23 and p38 MAPK were increased. When these hepatocytes were released by collagenases and were grown under the conditions of gene therapy grade tissue culture system, the enlarged nucleoli were further enlarged. The B23 content was apin increased under in vitro conditions. From these experiments, it is clear that the hepatocytes possess approximately 100 fold flexibility of nucleolar capacity. It is suggested that thioacetamide enhances the ribosome genesis and exaggerates the nucleologenesis ability.

IMMUNOTOXICITY

Arts JH, Droge SC, Spanhaak S, Bloksma N, Penninks AH, Kuper CF. **Local lymph node activation and IgE responses in brown Norway and Wistar rats after dermal application of sensitizing and non-sensitizing chemicals**. Toxicology 1997;117(2-3):229-34.
The local lymph node assay (LLNA) and the IgE test in the mouse are proposed models for predictive recognition of low molecular weight chemicals causing IgE-mediated allergic airway reactions in man. Since rats are commonly used in routine toxicity studies and a previous study (Arts et al. (1996) Food Chem. Toxicol. 34, 55-62) has shown that several rat strains were found appropriate for the LLNA, the suitability of the rat for the IgE test was examined in the present study. Serum IgE concentrations were examined following topical exposure of Brown Norway (BN) and Wistar rats to each of four chemicals with known diverse sensitization potential in humans: trimellitic anhydride (TMA), a dermal and respiratory sensitizer, dinitrochlorobenzene (DNCB), a dermal sensitizer with no or limited potential to cause respiratory allergy; formaldehyde (FA), a skin irritant and dermal sensitizer with equivocal evidence for respiratory sensitizing potential; methyl salicylate (MS), a skin irritant devoid of sensitizing properties. Of the four tested chemicals, only exposure to TMA resulted in a significant increase in serum IgE concentration and this response was only evoked in the high-IgE-responding BN rat. The latter two chemicals were also tested for lymph node activation, in casu the ear-draining lymph nodes. FA caused a dose-dependent activation of the draining lymph nodes whereas MS was inactive. The results as obtained with TMA, DNCB and MS in the rat are in agreement with human data. The results with FA though, indicate the need for further studies of chemicals that have both irritant and sensitizing properties at about similar concentrations or may act through non-IgE-mediated immune mechanisms.

Basketter DA, Gerberick GF. **An interlaboratory evaluation of the Buehler test for the identification and classification of skin sensitizers**. Contact Dermatitis 1996;35(3):146-51.
The correct identification of potential skin sensitizers is an essential first step in enabling a proper risk assessment to be made and to permit the implementation of appropriate risk management practices designed to avoid the induction of sensitization. Consequently, regulatory guidelines around the world demand that new substances are evaluated to assess their skin sensitization potential. There are two guinea pig test methods which are generally recognised, the guinea pig maximisation test (GPMT) and the occluded patch test described by Buehler. In different countries, one procedure seems to be more prevalent and acceptable to regulatory authorities than the other. Notably, in the European Union, the latest revision of the Annex V (Directive 92/32/EC) Test Method for skin sensitization asks that justification should be given in the situation where the notifier does not use the GPMT, which is the preferred method. Thus in this paper, the validity of the Buehler protocol in the context of European legislation is critically examined. Results from two laboratories are collated, showing that the method can identify significant contact allergens, particularly those which would be registered formally as such according to European legislation. It is demonstrated that minor methodological variations can be tolerated without compromising test sensitivity, but it is recommended that suitable positive control testing is the best way to ensure proper test conduct.

Dearman RJ, Smith S, Basketter DA, Kimber I. **Classification of chemical allergens according to cytokine secretion profiles of murine lymph node cells**. J Appl Toxicol 1997;17(1):53-62.
BIOSIS COPYRIGHT: BIOL ABS. Characteristic cytokine secretion profiles, consistent with the selective activation of discrete functional subpopulations of T helper (Th) cells, have been demonstrated following repeated topical exposure of mice to chemical contact or respiratory allergens. Draining lymph node cells (LNC) derived from animals treated with the respiratory allergen trimellitic anhydride (TMA; 10%) expressed high levels of the Th2 cytokines interleukins 4 and 10, but little of the Th1 cell product interferon gamm Under conditions of exposure of equivalent immunogenicity with respect to LNC proliferation, the contact allergen 2,4-dinitrochlorobenzene (DNCB) provoked the converse pattern of cytokine secretion. The purpose of the present investigations was to examine dose-response relationships with respect to cytokine production with a wider range of chemical allergens. In each

case, cytokine secretion patterns were compared with those observed with LNC prepared from animals exposed concurrently to TMA or DNCB. Despite some inter-experimental variation in the absolute amounts of cytokines produced, DNCB- and TMA-activated LNC invariably expressed Th1- and Th2-type patterns, respectively. At all concentrations tested, the contact allergens isoeugenol and formaldehyde stimulated a Th1-type cytokine secretion profile, whereas a Th2-type pattern was induced following exposure to the chemical respiratory allergens cyanuric chloride and diphenylmethane diisocyanate. These data demonstrate that divergent cytokine secretion profiles characterize immune responses to different classes of chemical allergen and suggest that it may be possible, in a single integrated assay, to identify and classify chemical allergens as a function of induced cytokine production patterns.

Descotes J, Patriarca C, Vial T, Verdier F. **The popliteal lymph node assay in 1996**. Toxicology 1997;119(1):45-9.
The popliteal lymph node (PLN) assay is based on the assumption that a mechanism similar to a graft-versus-host (GvH) reaction is involved in 'GvH-like' drug-induced side-effects, including generalized lymphadenopathy, serum sickness-like disease, scleroderma-like reaction and the lupus syndrome. An increased PLN weight 7-10 days after injection of the test article into the footpad is generally held as a positive response. Most, if not all compounds reported to induce pseudo-GvH side-effects in man (namely positive model compounds) have been shown to induce positive PLN responses in mice and/or rats. Reproducible results have been obtained in several laboratories, in some instances blindly. However, positive responses have also been obtained with the negative model compounds acetone and imipramine. Flow cytometry analysis and conventional histology failed to help differentiate between a true GvH response and a primary irritative effect. In order to confirm the potential value of the PLN assay to predict the risk for drug-induced GvH-like reactions, mechanistic studies are urgently needed.

Goebel C, Griem P, Sachs B, Bloksma N, Gleichmann E. **The popliteal lymph node assay in mice: screening of drugs and other chemicals for immunotoxic hazard**. Inflamm Res 1996;45(Suppl 2):85-90.
The popliteal lymph node assay (PLNA) in mice represents a predictive test for assessing the sensitizing (allergenic and autoimmunogenic) potential of drugs and low molecular weight chemicals. Measuring activation of the draining lymph node of the hind paw, the PLNA facilitates the detection and analysis of immunotoxic effects in a rapid and reproducible manner. An attractive feature of the PLNA is that it can be performed in combination with the routine toxicity testing required for new drugs. Thus, it is possible to investigate whether animals exposed by the oral, intravenous, or inhalative route have been sensitized to the test compound or a reactive metabolite of the test compound generated in vivo. PLNAs may be appropriate supplements to routine toxicity screening of chemicals, thereby enhancing chemical safety.

Hasseus B, Wallstrom M, Osterdahl BG, Hirsch JM, Jontell M. **Immunotoxic effects of smokeless tobacco on the accessory cell function of rat oral epithelium**. Eur J Oral Sci 1997;105(1):45-51.
BIOSIS COPYRIGHT: BIOL ABS. Smokeless tobacco (ST) is known to adversely effect the oral mucosa, but knowledge about the influence on immune defence is limited. Few studies have investigated the effect of ST on the local immune response. In the present study, we have assessed the effect of a crude Swedish moist snuff (SS) extract, alkaloids, and nitrosamines on T-cell mitogenic response to Con A using epithelial cells, including Langerhans cells, of the rat oral mucosa as accessory cells. SS extract at a concentration of 4% reduced the T-cell proliferation by 50% (IC50 = 4%). Pretreatment of either oral epithelial cells or T-cells with SS extract also gave a significant inhibition of T-cell proliferation. This effect was not obtained following preincubation with SS components as alkaloids and different tobacco-specific nitrosamines (TSNA). None of the tested compounds were found to possess any mitogenic properties. This in vitro study showed that SS extract can evoke an immunosuppressive effect on mitogen-driven T-cell proliferation using cells from oral epithelium as accessory cells. This effect was more pronounced when SS extract was employed compared to when the single SS components were used alone.

Kouchi Y, Maeda Y, Morinaga H, Ohuchida A. [**Immunotoxic effects of a new antineoplastic agent S-1 in mice--comparison with S-1, UFT and 5-FU**]. J Toxicol Sci 1996;21(Suppl 3):691-701. (Jpn)
The immunotoxicity of S-1, which is a new antineoplastic agent, was investigated in BALB/c mice. S-1 contains

tegafur (FT), CDHP, and potassium oxonate (Oxo) in a molecular ratio of 1:0.4:1. 5-fluorouracil (5-FU) and UFT were used as reference drugs. S-1 and reference drugs were administered by oral gavage for 7 days. The high dose employed in this study was determined as the maximally tolerated dose of a 9-day repeated-dose study in sarcoma 180-bearing mice. Decreased body weight was observed in mice treated with 5-FU and UFT but not in those treated with S-1. A significant decrease in thymus and spleen weight was observed in S-1-, UFT- and 5-FU-treated mice, and the degree was same for the three drugs. Though the number of white blood cells decreased dose-dependently for the three drugs, S-1 had the weakest effect. The number of red blood cells also decreased, but the effect was not dose-dependent, and its magnitude was the same for the 3 drugs. S-1 induced a dose-dependent decrease in the IgM antibody PFC response to sheep erythrocytes. The delayed type hypersensitivity response used a footpad reaction method was significantly suppressed at the highest dose of S-1. 5-FU and UFT suppressed humoral and cell-mediated immunity in almost the same manner as S-1. The degree of suppressive effects was greater on the humoral immune response than on the cell-mediated immune response. The number of CFU-GM colonies was significantly decreased in the highest dose group of each drug and in a lower group as well in S-1-treated mice. This finding might reflect the fact that S-1 induced continuous high levels of 5-FU in the blood. Under these experimental conditions, S-1 induced immunosuppressive effects in BALB/c mice, and the degree of suppression was almost same as that induced by 5-FU and UFT.

Kouchi Y, Maeda Y, Ohuchida A, Ohsawa M. **Immunotoxic effect of low dose cisplatin in mice**. J Toxicol Sci 1996;21(4):227-33.
The immunosuppressive effects of cisplatin at relatively low doses were investigated in CD-1 mice. Mice were injected intraperitoneally with 8, 40 and 200 micrograms/kg cisplatin for 10 days. A decrease in body and thymus weights was observed at 200 micrograms/kg. Though there were no dose-related effects on the IgM antibody response to sheep erythrocytes, a statistically significant reduction of the contact hypersensitivity response (CHR) was seen at 200 micrograms/kg. In vivo and in vitro effects of cisplatin on T- and B-lymphocyte function were assessed by proliferative response to concanavalin A and lipopolysaccharide, respectively. Cisplatin inhibited splenic T-lymphocyte function more than splenic B-lymphocyte function. These data indicate that a relatively low dose of cisplatin induce immunosuppressive effects in mice with a greater effect on T-lymphocytes than the B-lymphocytes.

Krasteva M, Peguet-Navarro J, Moulon C, Courtellemont P, Redziniak G, Schmitt D. **In vitro primary sensitization of hapten-specific T cells by cultured human epidermal Langerhans cells--a screening predictive assay for contact sensitizers**. Clin Exp Allergy 1996;26(5):563-70.
BACKGROUND: The need to develop predictive tests which could identify potential allergens has been recognized for many years. There is as yet no accepted in vitro method for the assessment of contact ensitizers. OBJECTIVE: We have tested the ability of a range of contact allergens to induce in vitro primary sensitization of autologous T cells. METHOD: T-cell proliferation induced by haptens using 2-day cultured human Langerhans cells as antigen-presenting cell was assessed by 3H thymidine incorporation. Antigen specific stimulation was calculated as stimulation indexes. RESULTS: Strong allergens induced in vitro a primary T-cell response in all (trinitrophenyl, TNP: 13/13) or in the majority (fluorescein isothiocyanate, FITC: 7/10) of experiments. An irritant, sodium dodecyl sulfate (SDS), failed to generate a significant T-cell proliferation in any of the experiments (0/10). We obtained a significant lymphoproliferative response to weak sensitizers only in a limited number of experiments: (coumarin: 1/12, citronellal: 0/10, hydroxycitronellal: 2/8). p-Phenylenediamine (PPDA), a prohapten and highly sensitizing chemical in vivo, generated primary sensitization in vitro in only one of six experiments, while Bandrowski's base (BB), a metabolization product of PPDA induced a significant T-cell response in all six experiments. CONCLUSION: The present in vitro model allows discrimination between two groups of substances: strong contact sensitizers (TNP, FITC, BB) on the one hand and weak sensitizers (coumarin, citronellal and hydroxycitronellal) and irritants (SDS) on the other hand. It could be used as a screening in vitro assay to eliminate strong contact allergens before further predictive animal tests have to be performed.

Sikorski EE, Gerberick GF, Ryan CA, Miller CM, Ridder GM. **Phenotypic analysis of lymphocyte subpopulations in lymph nodes draining the ear following exposure to contact allergens and irritants**.

Fundam Appl Toxicol 1996;34(1):25-35.
The murine local lymph node assay (LLNA) measures in vivo proliferation in draining lymph nodes (DLN) following topical exposure to chemicals to assess contact sensitization potential. However, proliferation has also been observed with some irritants. To further characterize events in the DLN during the LLNA and distinguish allergens from irritants, phenotypic analysis of lymphocyte subsets was made following topical exposure. In preliminary studies, mice were treated on the ears for 3 consecutive days, and 48 hr following the final application, analysis of CD3, CD4, CD8, and B220 expression was evaluated by flow cytometry. The allergens oxazolone (OXAZ) and picryl chloride (TNCB) and the irritant benzalkonium chloride (BC) increased cell number compared to vehicle. The increase in lymph node cellularity for these materials was due to an increase in the total number of T and B lymphocytes. Interestingly, even though contact sensitization is a cell-mediated immune response (Th1), mice exposed to the contact allergens showed a preferential increase in B lymphocytes in the DLN as seen by an increase in the percentage of B220+ cells. The percentage of B220+ cells was 13.1 and 36.1% for OXA and TNCB, respectively, compared to percentages of 7.4 and 9.3% for irritant and vehicle, respectively. With some allergens, a concomitant decrease in the percentage of CD3+ cells was seen. Time course studies demonstrated the increase in the percentage of B220+ cells was seen in allergen treated mice by 24 hr after the final application of material, plateaued by 48 hr, and was still elevated by 96 hr. In allergen-treated mice, percentages of B220+ cells increased dose dependently. Further studies were performed to evaluate additional contact allergens and irritants and determine if evaluation of flow cytometric parameters could potentially identify contact allergens and differentiate them from irritants. Analysis of data from these studies, which examined a total of five contact allergens and six irritants, showed that the modifications to the LLNA improved the identification of irritants and allergens in individual experiments by including both phenotypic analysis of the DLN and cell number per node as endpoints rather than either endpoint alone.

Vohr HW. **Experiences with an advanced procedure for the identification of chemicals with an immunotoxic potential in routine toxicology**. Toxicology 1995;104(1-3):149-58.
BIOSIS COPYRIGHT: BIOL ABS. The development or selection of suitable tests for immunotoxicological screening and thus for incorporation into guidelines presents some problems. Most of the tests which have been proposed for immunotoxicological investigations and most knowledge and experience in immunology are based on mouse models. The standard species in the early phase of toxicological testing, however, is the rat. Any discussion about basic tests is hampered by a paucity of data from routine toxicological and/or epidemiological studies. Here we present data obtained from an advanced screening battery on the basis of OECD guideline 407. Thirteen pesticides of early developmental stage had been included in this screening. Two out of these 13 compounds turned out to be cytotoxic and were picked up by the immunological parameters as being 'primary immunotoxic', i.e., immunological changes not induced by overtly toxic doses ('indirect or secondary immunotoxic'). The advantages and disadvantages of each additional test is discussed as well as the comparison of the results obtained on the basic and the extended guideline test battery. In summary, the tests described here show that a little extra effort at the screening stage can save animals, time and costs for additional testing.

Woods KM, Nesterenko MV, Upton SJ. **Efficacy of 101 antimicrobials and other agents on the development of Cryptosporidium parvum in vitro**. Ann Trop Med Parasitol 1996;90(6):603-15.
An in-situ ELISA was used as a primary screen to test the effects of 101 antimicrobials and other agents on the development of Cryptosporidium parvum in vitro. Over 40 of the compounds displayed some form of anticryptosporidial activity, and dose-response curves were generated for 40 of these. The in-situ ELISA makes a highly effective primary, pharmaceutical screen for C parvum, to be used prior to more detailed microscopical, toxicological or in-vivo assays.

NEUROTOXICITY

Bruinink A, Rasonyi T, Sidler C. **Reduction of ochratoxin A toxicity by heat-induced epimerization. In vitro effects of ochratoxins on embryonic chick meningeal and other cell cultures**. Toxicology 1997;118(2-3):205-10.
The widespread contamination of food by mycotoxins may present a serious hazard to human and animal health. The present study was designed to determine the toxic potential of three structurally related ochratoxins: ochratoxin A (OTA), ochratoxin B (OTB) and the heat-induced 3S-epimer of OTA (3S-OTA) recently discovered in roasted coffee and human serum. The toxicity was determined using serum-free cell cultures of embryonic chick meningeal fibroblasts, taking the effects on mitochondrial and lysosomal activity and culture protein content as an index for toxicity. OTA, OTB and 3S-OTA were toxic. However, the concentration necessary to induce comparable effects were nearly 19- and 10-fold higher for OTB and 3S-OTA, respectively, than those for OTA. In a next step the sensitivity of serum-free cell cultures of embryonic chick neural retina and brain were compared in relation to meningeal cell cultures. In the present study, no indications for differences in sensitivity could be detected. Furthermore, our study suggest that the OTA-induced toxic effects are not due to the inhibition by OTA of phenylalanine-tRNA synthetase.

Bruinink A, Sidler C, Birchler F. **Neurotrophic effects of transferrin on embryonic chick brain and neural retinal cell cultures**. Int J Dev Neurosci 1996;14(6):785-95.
The viability and differentiation promoting effects of various transferrins [iron-saturated (holo) and iron-depleted (apo) human and chick ovo (conalbumin)-transferrins, and bovine apo-transferrin] were studied, using serum-free, flat-sedimented cell cultures of embryonic chick brain and neural retina. The effects of transferrin (Tf) on the cell cultures depended on the type of Tf used and the parameter measured. Significant differences between brain and neural retina cultures in the effects of apo-ovoTf and iron [supplemented as ammonium-iron (III) citrate] were detected. Maximal levels of mitochondrial activity were observed in the presence of 2 mg/l apo-ovoTf in neural retina cell cultures. In brain cell cultures, 40 mg ovoTf/l were needed to achieve maximal levels. In brain, but not in neural, retina cell cultures ovoTf and optimal concentrations of Fe3+ exhibited similar effects on biochemical parameters of cell function and differentiation. Although, in the absence of ovoTf, neuronal outgrowth on areas not covered by glial cells was inhibited in both cell cultures, the differences were more prominent in neural retina cell cultures. Our data strongly suggest that Tf plays a key role in processes not connected directly with its iron transport capability.

Chen JC, Fine RE, Squicciarini J, Volicer L. **Neurotoxicity of free-radical-mediated serotonin neurotoxin in cultured embryonic chick brain neurons**. Eur J Pharmacol 1996;303(1-2):109-14.
Exposure of serotonin (5-HT) to oxygen-derived free-radical-generating system, xanthine oxidase-hypoxanthine or to a Fenton reaction results in the formation of the neurotoxin, tryptamine-4,5-dione. In cultured embryonic chick brain neurons, incubation of tryptamine-4,5-dione or its ethyl carbonate derivative resulted in a dose-dependent neurotoxicity (1-100 microM). The addition of sulfhydryl compound, glutathione at 2 or 10 microM significantly enhanced the toxicity induced by 10 microM tryptamine-4,5-dione. On the contrary, glutathione at 10 microM decreased the neurotoxic effect caused by 10 microM 5,6- and 5,7-dihydroxytryptamine in the cultured neurons. The toxicity resulted from 5,6- and 5,7-dihydroxytryptamine could be fully prevented by a 5-HT uptake inhibitor, fluoxetine. However, the toxicity caused by tryptamine-4,5-dione and glutathione conjugate could not be blocked by fluoxetine (10 or 100 microM) or by a glutathione transferase inhibitor, boric acid/serine. The results indicate a different molecular mechanism among 5-HT derived neurotoxins and suggest that tryptamine-4,5-dione and/or its glutathione conjugate would cause neuronal damage, if they are formed in vivo.

Debont T, Daenens P, Tytgat J. **An improved fractionation and fast screening method for the identification of new and selective neurotoxins**. Neurosci Res 1996;24(2):201-6.
Neurotoxins have highly specific actions on molecular targets, and thus offer an effective means of characterizing the growing number of identified ion channels and receptors in the nervous system. Separation procedures leading to the identification of neurotoxins almost always include gel filtration chromatography, combined with ion-exchange and/or reversed phase chromatography. We present here an improved fractionation method based on the use of a new Superdex 30 prep grade HiLoad 16/60 FPLC gel filtration column. This single-step gel filtration protocol results

in a shortening of the purification process and allows a superior qualitative separation of (neuro-)peptides in crude venoms as compared to any other type of gel filtration column used thus far. Screening of the collected fractions for potential ion channel blocking properties was performed by means of the whole-cell voltage clamp technique. To increase both the amount and speed of expression in Xenopus laevis oocytes of cloned ion channels, we employed a high-expression vector, pGEMHE, wherein the cDNA encoding a neuronal voltage-dependent potassium channel (RCK1) was subcloned. The combination of these techniques represents a fast and efficient identification procedure in the quest for new and selective neurotoxins for cloned channels and receptors.

Dickie BG, Holmes C, Greenfield SA. **Neurotoxic and neurotrophic effects of chronic N-methyl-D-aspartate exposure upon mesencephalic dopaminergic neurons in organotypic culture**. Neuroscience 1996;72(3):731-41.
Current theories regarding the mechanisms of degeneration of dopaminergic nigrostriatal neurons in Parkinson's disease suggest a pivotal role for excitotoxicity. In this study, the effects of chronic exposure of rat ventral mesencephalic slice cultures to the excititoxin N-methyl-D-aspartate, were investigated. Chronic (18 day) exposure to N-methyl-D-aspartate produced widely varying, dose-dependent effects. High doses (100 mu M) caused a pronounced toxicity upon tyrosine hydroxylase-positive neurons, with the surviving neurons possessing shrunken somata and stunted neurites: co-administration of the N-methyl-D-aspartate receptor antagonist MK-801, inhibited N-methyl-D-aspartate-induced toxicity. In contrast, exposure to a low concentration of N-methyl-D-aspartate (0.1 mu M), stimulated the outgrowth of tyrosine hydroxydase-positive neurites from the culture; this effect was abolished by MK-801. Chronic application of glutamate had similar, though not as pronounced, growth-promoting actions. However, the concentration of glutamate required was 1000 times that of N-methyl-D-aspartate, due to the presence ot high-affinity glutamate transport mechanisms. Cultures exposed to a submicromolar concentration of N-methyl-D-aspartate exhibited a significant resistance to subsequent exposure to a lethal (300 mu M) concentration of the toxin. It would thus appear that N-methyl-D-aspartate may have both trophic and toxic actions upon dopaminergic neurons in culture. Moreover, the ability of low doses of N-methyl-D-aspartate to protect neurons in this critical brain region may be of relevance to future attempts to arrest the degeneration associated with Parkinson's disease. The putative mechanisms of these phenomena are discussed.

Dunigan CD, Shamoo AE. **Identification of the major transport pathway for the parkinsonism-inducing neurotoxin 1-methyl-4-phenylpyridinium**. Neuroscience 1996;75(1):37-41.
1-Methyl-4-phenylpyridinium is a potent parkinsonism-inducing neurotoxin which has become a valuable tool for the examination of the mechanisms and therapeutic treatment strategies for Parkinson's syndrome. Recently, it has been found that physiological levels of extracellular ATP (0.1-1 mM) stimulate dopamine uptake into both rat and bovine brain synaptosomes and rat pheochromocytoma cells in a dose-dependent manner. In this study we report that physiological levels of extracellular ATP (0.1-2 mM) stimulate the transport of 1-methyl-4-phenylpyridinium into the pheochromocytoma cell line by 270% over basal levels. Kinetically, the presence of ATP increases both the K (m) and Vmax of 1-methyl-4-phenylpyridinium transport. In addition, 1-methyl-4-phenylpyridinium is far more effective at inhibiting ATP-stimulated dopamine transport (IC50 = 11 microM) than basal dopamine transport (IC50 100 microM) into pheochromocytoma cells. These data show that the ATP-regulated 1-methyl-4-phenylpyridinium transport pathway is the major component (approximately 95%) of total 1-methyl-4-phenylpyridinium transport, and provide the first evidence for the involvement of extracellular ATP in the bulk transport of 1-methyl-4-phenylpyridinium.

Eisch AJ, O'dell SJ, Marshall JF. **Striatal and cortical NMDA receptors are altered by a neurotoxic regimen of methamphetamine**. Synapse 1996;22(3):217-25.
Methamphetamine (m-AMPH) treatment produces long-lasting damage to striatal and cortical monoaminergic terminals and may also injure nonmonoaminergic cortical neurons. Evidence suggests that both dopamine (DA) and glutamate (GLU) play crucial roles in producing this damage. We used quantitative autoradiography to examine [3H] mazindol ([3H]MAZ) binding to striatal DA transporters and [3H]GLU binding to N-methyl-D-aspartate (NMDA) receptors in the striatum and cortex 1 week and 1 month after a neurotoxic regimen of m-AMPH. Rats received m-AMPH (4 mg/kg) or saline (SAL) (1 ml/kg) in four s.c. injections separated by 2 h intervals. One week after m-

AMPH, the ventral and lateral sectors of the striatum showed the greatest decreases in both [3H]MAZ and [3H]GLU binding, while the nucleus accumbens (NA) showed no significant decreases. One month after m-AMPH, striatal [3H]MAZ binding was still significantly decreased, while NMDA receptor binding had recovered. Surprisingly, the parietal cortex showed a m-AMPH-induced increase in NMDA receptor binding in layers II/III and IV 1 week after m-AMPH and only in layers II/III 1 month after m-AMPH. The prefrontal cortex showed no m-AMPH-induced changes in NMDA receptor binding at either time point. This is the first demonstration that a regimen of m-AMPH that results in long-lasting damage to DA terminals can alter forebrain NMDA receptor binding. Thus, repeated m-AMPH treatments may produce changes in glutamatergic transmission in selected striatal and cortical regions.

Fernandez AM, Garcia-Estrada J, Garcia-Segura LM, Torres-Aleman I. **Insulin-like growth factor I modulates c-Fos induction and astrocytosis in response to neurotoxic insult**. Neuroscience 1997;76(1):117-22.
Insulin-like growth factor I participates in the cellular response to brain insult by increasing its messenger RNA expression and/or protein levels in the affected area. Although it has been suggested that insulin-like growth factor I is involved in a variety of cellular responses leading to homeostasis, mechanisms involved in its possible trophic effects are largely unknown. Since activation of c-Fos in postmitotic neurons takes place both in response to insulin-like growth factor I and after brain injury, we have investigated whether this early response gene may be involved in the actions of insulin-like growth factor I after brain insult. Partial deafferentation of the cerebellar cortex by 3-acetylpyridine injection elicited c-Fos protein expression on both Purkinje and granule cells of the cerebellar cortex. This neurotoxic insult also triggered gliosis, as determined by an increased number of glial fibrillary acidic protein-positive cells (reactive astrocytes) in the cerebellar cortex. When 3-acetylpyridine-injected animals received a continuous intracerebellar infusion of either a peptidic insulin-like growth factor I receptor antagonist or an insulin-like growth factor I antisense oligonucleotide for two weeks through an osmotic minipump, c-Fos expression was obliterated while reactive gliosis was greatly increased. On the contrary, continuous infusion of insulin-like growth factor I significantly decreased reactive gliosis without affecting the increase in c-Fos expression. These results indicate that insulin-like growth factor I is involved in both the neuronal (c-Fos) and the astrocytic (glial fibrillary acidic protein) activation in response to injury.

Kilburn KH. **Chlordane as a neurotoxin in humans**. South Med J 1997;90(3):299-304.
BIOSIS COPYRIGHT: BIOL ABS. To assay and profile chronic neurobehavioral impairment associated with chlordane exposure in symptomatic patients, consecutive evaluations of nine patients were done with sensitive neurophysiologic and neuropsychologic tests for neurobehavioral function. Their fields, balance, reaction time, blink, color discrimination, grip strength, cognitive function, recall, memory, and perceptual motor speed were tested, and mood states and frequencies of 35 symptoms were appraised. Prevalences of abnormality were compared test-by-test to predict values with confidence intervals, and mean values for the group were compared with reference values. Testing showed abnormal balance with eyes dosed in 7, abnormal color discrimination in 6, verbal recall deficit in 5, and prolonged blink reflex latency, prolonged choice reaction time, and decreased Culture Fair scores in 4 each. Profile of Mood States score was elevated in 5. These observations suggest that chlordane causes protracted neurotoxicity.

Koh DS, Hille B. **Modulation by neurotransmitters of catecholamine secretion from sympathetic ganglion neurons detected by amperometry**. Proc Natl Acad Sci U S A 1997;94(4):1506-11.
CBAC COPYRIGHT: CHEM ABS Many neuromodulators inhibit N-type Ca2+ currents via G protein-coupled pathways in acutely isolated superior cervical ganglion (SCG) neurons. Less is known about which neuromodulators affect release of norepinephrine (NE) at varicosities and terminals of these neurons. To address this question, the authors used carbon fiber amperometry to measure catecholamine secretion evoked by elec. stimulation at presumed sites of high terminal d. in cultures of SCG neurons. The pharmacol. properties of action potential-evoked NE release paralleled those of N-type Ca2+ channels: Release was completely blocked by Cd2+ or omega-conotoxin GVIA, reduced 50% by 10 muM NE or 62% by 2 muM UK-14,304, an alpha2-adrenergic agonist, and reduced 63% by 10 muM oxotremorine M (Oxo-M), a muscarinic agonist. Consistent with action at M2 or M4 receptor subtypes, Oxo-M could be antagonized by 10 muM muscarinic antagonists methoctramine and tropicamide but not by pirenzepine. After overnight incubation with pertussis toxin, inhibition by UK-14,304 and Oxo-

M was much reduced. Other neuromodulators known to inhibit Ca2+ channels in these cells, including adenosine, prostaglandin E2, somatostatin, and secretin, also depressed secretion by 34-44%. In cultures treated with omega-conotoxin GIA, secretion dependent on L-type Ca2+ channels was evoked with long exposure to high K+ Ringer's soln. This secretion was not sensitive to UK-14,304 or Oxo-M. Evidently, many neuromodulators act on the secretory terminals of SCG neurons, and the depression of NE release at terminals closely parallels the membrane-delimited inhibition of N-type Ca2+ currents in the soma.

Makhaeva GF, Filonenko IV, Malygin VV. [**Comparative studies of interaction of dichlorovinyl esters of phosphoric acids with hen and rat brain neurotoxic esterase**]. Zh Evol Biokhim I Fiziol 1995;31(4):396-403. (Rus)
BIOSIS COPYRIGHT: BIOL ABS. In order to validate a rodent biochemical model of delayed neurotoxicity of organophosphates (OP) inhibition of rat and hen brain neurotoxic esterase (NTE) by some dichlorovinyl phosphates and phosphonates was studied in vitro and in vivo. It was shown that compounds investigated exhibited the similar inhibitory potency to NTE from both species in vitro, in addition rat and hen NTE showed the same sensitivity to variation of the structure of OP inhibitors. A good correlation was found between pI50 estimated with enzymes from rat and hen trains: r2 = 0.951, n = 18, p : 0.05. NTE activities were also measured in rat and hen brains after acute administration of various dosages of potent axonopathic compound dipropyldichlorovinyl phosphate. The results obtained indicate that difference in species susceptibility to neurotoxic action of OP, in particular the absence of ataxia in rats, is not caused by difference in target enzyme sensitivity to axonopathic organophosphates.

Maruyama W, Sobue G, Matsubara K, Hashizume Y, Dostert P, Naoi M. **A dopaminergic neurotoxin, 1(R), 2(N)-dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, N-methyl(R)salsolinol, and its oxidation product, 1,2 (N)-dimethyl-6,7-dihydroxyisoquinolinium ion, accumulate in the nigro-striatal system of the human brain**. Neurosci Lett 1997;223(1):61-4.
BIOSIS COPYRIGHT: BIOL ABS. N-Methyl(R)salsolinol was found to be an endogenous dopaminergic neurotoxin inducing parkinsonism in rodents and to increase in the cerebrospinal fluid of parkinsonian patients. The amounts of N-methyl(R)salsolinol and related compounds in the human brain regions were quantitatively analyzed. Only the (R)-enantiomer of salsolinol derivatives were detected, which suggests their enzymatic synthesis in situ. In the nigro-striatal system, the concentration of N-methyl(R)salsolinol was higher than in the frontal cortex, and its oxidized catechol isoquinolinium ion was detected only in the substantia nigra significantly. The accumulation of these neurotoxins in the nigro-striatal region might account for selective cell death of dopamine neurons in the substantia nigra of Parkinson's disease.

Matsuoka M, Matsumura H, Igisu H. **Creatine kinase activities in brain and blood: possible neurotoxic indicator of acrylamide intoxication**. Occup Environ Med 1996;53(7):468-71.
The possible correlation between neurological disturbances in mice given acrylamide (79061) and creatine-kinase (CK) activity in the brain was investigated. Male ddy-mice were injected intraperitoneally with acrylamide in saline at 50mg/kg/day from day zero through seven. Mice were sacrificed on day five, eight, 11, 15, 23, 36, or 50. In a second study male Wistar-rats were given either acrylamide or isotonic saline using the same procedure. Rats were sacrificed 24 hours after the last injection and blood collected by cardiac puncture to study the activities of plasma aspartate-aminotransferase, alanine-aminotransferase, alkaline-phosphatase, lactate-dehydrogenase (LDH), cholinesterase, and CK. CK activity was suppressed by acrylamide in the brain of the mice in parallel with the neurological dysfunction measured by landing foot spread. There were no clear alterations in the glyceraldehyde-3-phosphate dehydrogenase, neuron specific enolase, and lactate-dehydrogenase activities over the study period. Among the plasma enzymes studied, suppression of CK was most notable, but thyroid activity was not affected.

Randall JC, Ambroso JL, Groutas WC, Brubaker MJ, Richardson RJ. **Inhibition of neurotoxic esterase in vitro by novel carbamates**. Toxicol Appl Pharmacol 1997;143(1):173-8.
Carbamyl sulfonate (CS) compounds are a novel class of carbamates derived from amino acid methyl esters. They have the general structure RCH(COOCH3)NH(CO)SO-3K+, where R is the sidechain of the parent amino acid.

These compounds were developed as active site-directed inhibitors of human leukocyte elastase (HLE). The purpose of this study was to characterize the inhibition of hen brain neurotoxic esterase (neuropathy target esterase, NTE), horse serum butyrylcholinesterase (BuChE), and bovine erythrocyte acetylcholinesterase (AChE) by CS analogs derived from the methyl esters of L-ala, D-norval, L-norval, L-phe, L-val, L-norleu D-met, and L-met. Bimolecular rate constants of inhibition (ki) for NTE ranged from 0.571 for L-ala-CS to 17.7 mM-1 min-1 for L-norleu-CS (10-min I50 values of 123 and 3.92 microM, respectively). Potency against NTE increased with chain length for straight-chain R-groups of L-CS compounds. Unlike HLE, NTE was only weakly stereoselective for CS compound enantiomers. The L-isomers were weaker inhibitors of BuChE than NTE (10-min I50 range of 742 to 35.6 microM). In contrast to the L-enantiomers, the I50 plots of D-met-CS and D-norval-CS were not linear for BuChE, suggesting a possible stereospecific mechanistic shift for inhibition of this enzyme, AChE was not effectively inhibited by any of the CS compounds (I50 values > 750 microM). The specificity and charged nature of CS compounds give these unusual NTE inhibitors potential advantages for mechanistic studies of organophosphorus compound-induced delayed neurotoxicity (OPIDN) and its protection or potentiation.

Rossi J 3d, Ritchie GD, Macys DA, Still KR. **An overview of the development, validation, and application of neurobehavioral and neuromolecular toxicity assessment batteries: potential applications to combustion toxicology**. Toxicology 1996;115(1-3):107-17.
Currently, there are few alternatives to the use of animals in toxicology for human risk assessment. Neurobehavioral toxicology is an emerging area in which complex performance capacity is evaluated during or following toxicological exposure. While a number of single tests and a few more complex neurobehavioral batteries exist, no fully validated and comprehensive neurobehavioral toxicity assessment battery has yet been developed. The Neurobehavioral Toxicity Assessment Battery (NTAB) is a multi-test battery being developed by the Naval Medical Research Institute Detachment (Toxicology) (NMRI/TD) to categorize the potential neurobehavioral toxicity of compounds of Navy interest, especially those found in combustion atmospheres. The NTAB is intended to identify specific areas of deficit (e.g. motivational, sensory, motor, and cognitive) from complex changes in performance induced by toxic exposures, as well as to provide a mechanism to evaluate recovery of neurobehavioral integrity. Portions of the NTAB have been successfully used to assess the risk of brief exposure to low concentrations of combustion gases, including smoke from electrical aircraft fires, ozone-depleting substances and their replacements, and the novel neuroconvulsant trimethylolpropane phosphate. The goal of the NMRI/TD Neurobehavioral Toxicology Group and the Tri-Service Toxicology Consortium's neurobehavioral toxicology program is the incorporation of more molecular techniques involving neurophysiology, neuropharmacology, in vivo electrochemistry, and real-time microdialysis for correlative use with the neurobehavioral battery in human risk assessment. This overview discusses the application of neurobehavioral and neuromolecular endpoint test batteries to combustion toxicology.

Sakaki Y, Fukuda Y, Yamashita M. **Muscarinic and purinergic Ca2+ mobilizations in the neural retina of early embryonic chick**. Int J Dev Neurosci 1996;14(6):691-9.
Acetylcholine and adenosine triphosphate (ATP) raise intracellular Ca2+ concentration via muscarinic receptors and P2U purinoceptors by releasing Ca2+ from intracellular Ca2+ stores in the neural retina of early embryonic chick. The signal transduction mechanisms for the muscarinic and purinergic Ca2+ responses were studied with fura-2 fluorescence measurements. Li+ (1 mM), which inhibits phosphatidylinositol metabolism, enhanced both the Ca2+ rises to carbamylcholine (CCh. 30 microM) a muscarinic agonist and ATP (200 microM). Thapsigargin (250 nM), an inhibitor of Ca(2+)-ATPase of inositol trisphosphate (IP3)-sensitive Ca2+ stores, abolished both the Ca2+ rises to Cch (100 microM) and ATP (500 microM). U-73122 (2 microM), an inhibitor of phospholipase C beta, suppressed the Ca2+ rise to ATP (500 microM), but its analog U-73343 (2 microM) did not suppress it. In contrast, both U-73122 and U-73343 suppressed the Ca2+ the Ca2+ rise to CCh (100 microM). Pertussis toxin (250 ng/ml) suppressed the ATP-induced Ca2+ rise at least partly, whereas no inhibition was observed on the CCh-induced Ca2 + rise. Cross-talk occurred between the muscarinic and purinergic Ca2+ mobilizations but they were not occlusive. This study suggests that the muscarinic and purinergic Ca2+ mobilizations utilize IP3-sensitive Ca2+, stores, but different signal transduction pathways are involved in between the muscarinic and purinergic Ca2+ responses.

Sakaki Y, Sugioka M, Fukuda Y, Yamashita M. **Capacitative Ca2+ influx in the neural retina of chick embryo**. J

Neurobiol 1997;32(1):62-8.
Depletion of intracellular Ca2+ stores induces a capacitative Ca2+ influx in non-neural cells. It has been unknown whether the capacitative Ca2+ influx occurs in the cells of nervous systems. We found the capacitative Ca2+ influx in the neural retina of early embryonic chick with Fura-2 fluorescence measurements. A Ca(2+)-free medium containing thapsigargin (500 nM), an inhibitor of Ca(2+)-ATPase of intracellular Ca2+ stores, was applied to the neural retina of embryonic day 3 (E3) chick. A rise in intracellular Ca2+ concentration was evoked after the reintroduction of extracellular Ca2+, and this Ca2+ rise was suppressed by Zn2+ (1 mM) and Ni2+ (5 mM). The developmental changes in the Ca2+ rise induced by thapsigargin (250 nM) were studied from E3 to E13. The thapsigargin-induced Ca2+ rise was largest at E3, declined rapidly toward E6, and then decreased gradually until E13, when the Ca2+ rise almost disappeared. This developmental profile correlated with the decline in the mitotic activities of the retinal cells studied by Prada et al. The fluorescence imaging with the vertical slice of the E9 retina showed that the site at which the thapsigargin-induced Ca2+ rise was largest was the most outer layer of the retina, where proliferating cells are located. This spatial distribution and the above developmental profile may suggest that the capacitative Ca2+ influx occurs at the early period of neurogenesis when the cells have mitotic activities.

Schmuck G, Schluter G. **An in vitro model for toxicological investigations of environmental neurotoxins in primary neuronal cell cultures**. Toxicol Ind Health 1996;12(5):683-96.
Currently, most neurotoxicological investigations are still conducted using various animal models (e.g. chickens, rodents). In this report, alternative strategies of testing were examined to detect the neurotoxic potency of foreign compounds. Primary neuronal cell cultures from fetal rats are already an accepted model for mechanistic and pharmacological studies in drug research. Their suitability for neurotoxicological studies was examined by using industrial model compounds, which are well-known inductors of neuropathies: acrylamide, hexachlorophene, paraquat, n-hexane, and its neurotoxic metabolites acetylaceton and 2,5-hexandione. As a control compound, the nonneurotoxic solvent n-heptane was used. General cytotoxicity and the intracellular content of glial fibrillary acid protein, neuron-specific enolase, and neurofilaments were measured. n-Heptane induced an acute cytotoxicity and acrylamide and 2,5-hexandione produced a delayed cytotoxicity in primary neuronal cells, whereas the others showed no cytotoxic potency in the tested concentration range. These results were in agreement with the quantification of neurons by neuron-specific enolase. In contrast, with the exception of acetylaceton, glia cells were significantly affected by all neurotoxins at the later time. Signs of axonopathies were demonstrated for acrylamide, n-hexane and its metabolites, as well as for hexachlorophene and paraquat in vitro, by determining the intracellular neurofilament level. Therefore, the determination of cell-specific end points is necessary to detect the neurotoxic potency and quality of a compound, whereas the cytotoxicity assay limited the tested concentration range.

Stott WT, Beekman MJ, Johnson KA, Spencer PJ. **Evaluation of a novel assay of potential toxicity/ neurotoxicity of carpet emissions (VOCs) in mice**. Food Chem Toxicol 1997;35(2):241-54.
CBAC COPYRIGHT: CHEM ABS A private testing lab. utilizing the whole-body plethysmograph/head-only exposure app. outlined in the respiratory irritation assay ASTM E981-84, along with a novel exposure regimen, has reported neurotoxic effects and mortality in mice exposed to relatively low levels of volatile org. compds. (VOCs) emitted from a no. of consumer products. This methodol. was evaluated by exposing groups of mice, including unrestrained and sham-treated animals, to VOCs generated from a sample of carpet reported to be neurotoxic using the modified assay. General toxicol. (hematol. measurements, organ wts., gross pathol., histopathol.) and specific neurotoxicity (functional observations, body temp., histopathol. of nervous tissues) parameters were evaluated. No effects related to exposure to carpet VOCs were obsd. in the mice. However, despite careful handling, a no. of effects were obsd. which were attributed to the repeated restraint of mice in the ASTM E981 app. These included a no. of minor phys. injuries, decreased body wts., altered thymus wts., compression damage to the liver, and hemorrhage of the pituitary gland. Thus, the modification of the original ASTM E981 methodol. may result in phys. injuries and stress which may significantly affect any evaluation of toxicity and neurotoxicity in treated animals and result in inaccurate conclusions.

Tanaka S, Koike T. **Veratridine delays apoptotic neuronal death induced by NGF deprivation through a Na+-dependent mechanism in cultured rat sympathetic neurons**. Int J Dev Neurosci 1997;15(1):15-27.

CBAC COPYRIGHT: CHEM ABS Superior-cervical ganglion (SCG) cells dissocd. from newborn rats depend on nerve growth factor (NGF) for survival. Membrane depolarization with elevated K+ is known to prevent neuronal death following NGF deprivation and/or to promote survival via a Ca2+-dependent mechanism. This work studied the possibility of whether or not a Na+-dependent pathway for neuronal survival is present in these cells. Veratridine (EC50 40 nM), a voltage-dependent Na+ channel activator, delayed the onset of apoptotic cell death in NGF-deprived SCG neurons that had been cultured for 7 days in the presence of NGF. This effect was blocked completely by Na+ channel blockers, including tetrodotoxin (TTX, 1 muM), benzamil (25 muM) and flunarizine (1 muM), but was not attenuated by nimodipine (1 muM), an L-type Ca2+ channel blocker. The saving effect of veratridine on cultured neurons was obsd. even in low-Ca2+ media (0-1.0 mM), but was completely abolished in a low-Na+ medium (38 mM). Benzofuran isophthalate was employed as a fluorescent probe for monitoring the level of cytoplasmic free Na+, which revealed a sustained increase in its level (12.9 mM, 307% that of controls) in response to veratridine (0.75 .mu.M). TTX or flunarizine completely blocked the veratridine-induced Na+ influx in these cultured neurons. Moreover, no appreciable increase in intracellular Ca2+ was detected under these conditions. Though Na+ channels were active in SCG neurons freshly isolated from newborn rats, the Na+-dependent saving effect of veratridine was not obsd. in these young neurons. These lines of evidence suggest that the death-suppressing effect of veratridine on cultured SCG neurons depends on the Na+ influx via voltage-dependent Na+ channels, and suggests the presence of Na+-dependent regulatory mechanism(s) in neuronal survival.

Vaglini F, Pardini C, Cavalletti M, Maggio R, Corsini GU. **L-deprenyl fails to protect mesencephalic dopamine neurons and PC12 cells from the neurotoxic effect of 1-methyl-4-phenylpyridinium ion**. Brain Res 1996; 741 (1-2):68-74.
L-Deprenyl, a monoamine oxidase (MAO)-B inhibitor, appears to slow down the progression of Parkinson's disease. While inhibition of MAO-B activity can account for some of the effects of this substance, the basis by which L-deprenyl slows the progression of the disease remains controversial. In recent years, a new mechanism of action has emerged that may explain the ability of L-deprenyl to increase neuronal survival. L-deprenyl has been reported to modify gene expression and protein synthesis in astrocytes and PC12 cells. In this study, we tested the ability of L-deprenyl to protect mouse mesencephalic cells from the toxicity of the 1-methyl-4-phenyl pyridinium ion (MPP+). We exposed mouse mesencephalic cell cultures to L-deprenyl (10 microM) and, 24 h later, to MPP+ (2.5 microM). On the fifth day after L-deprenyl and MPP+ exposition, cells were washed free of drugs, and the following day they were tested for dopamine uptake, intracellular dopamine content and tyrosine hydroxylase immunoreactivity. The experiments were performed either in the presence or in the absence of glia. It was found that L-deprenyl pretreatment failed to achieve any protection against MPP+ toxicity. The fall in dopamine uptake and intracellular dopamine content, and the diminution of tyrosine hydroxylase immunoreactivity observed in cells pretreated with L-deprenyl and then given MPP+ were not significantly different from the values observed in cells treated with MPP+ alone. Additional experiments performed in PC12 cells, confirmed the failure of L-deprenyl to abolish the toxicity of MPP+. Our data seem to be at variance with previous reports demonstrating that the MAO-B inhibitor L-deprenyl protects dopaminergic neurons against MPP+ toxicity [12,20]; furthermore they do not support alternative mechanisms of action of L-deprenyl against MPP+ toxicity.

Yu B, Shinnick-Gallagher P. **Dihydropyridine- and neurotoxin-sensitive and -insensitive calcium currents in acutely dissociated neurons of the rat central amygdala**. J Neurophysiol 1997;77(2):690-701.
The central amygdala (CeA) is an area involved in emotional learning and stress, and identification of Ca2+ currents is essential to understanding interneuronal communication through this nucleus. The purpose of this study was to separate and characterize dihydropyridine (DHP)- and neurotoxin-sensitive and -resistant components of the whole cell Ca2+ current (ICa) in acutely dissociated rat CeA neurons with the use of whole cell patch-clamp recording. Saturating concentrations of nimodipine (NIM, 5 microM), a DHP antagonist, blocked 22% of ICa: this NIM-sensitive (L-type) current was recorded in 68% of CeA neurons. The DHP agonist Bay K 8644 (5 microM) produced a 36% increase in ICa in a similar proportion of CeA neurons (70%). omega-Conotoxin GVIA (CgTx GVIA, 1 microM) in saturating concentrations inhibited 30% of ICa, whereas omega-agatoxin IVA (Aga IVA, 100 nM), in concentrations known to block P-type currents, did not affect ICa. Higher concentrations of Aga IVA (1 microM) alone reduced ICa by 34%, but in the presence of NIM (5 microM) and CgTx GVIA (1 microM) blocked only 18% of ICa. omega-

Conotoxin MVIIC (CgTx MVIIC, 250 nM) reduced ICa by 13% in the presence of CgTx GVIA (1 microM). Application of NIM (5 mM), CgTx GVIA (1 microM); and Aga IVA (1 microM) blocked approximately 67% of ICa. A similar portion (63%) of Ca2+ current was blocked with CgTx MVIIC (250 nM) in the presence of NIM (5 microM) and CgTx GVIA (1 microM). The current resistant to NIM and the neurotoxins represented 37% of ICa, whereas in neurons not having L-type currents the resistant current made up approximately 53% of ICa (49 +/- 2%, mean +/- SE). The resistant current activated at around -40 mV and peaked at approximately 0 mV with half-activation and -inactivation potentials of -17 and -58 mV and slopes for activation and inactivation of -5 and 13 mV, respectively. The resistant current was sensitive to Cd2+ (IC50 = 2.5 microM) and Ni2+ (IC50 = 86 microM), was larger in Ca2+ than in Ba2+ (ratio = 1.31:1), and showed a moderate rate of decay. In summary, our results show that the high-voltage-activated calcium current in rat CeA neurons is composed of at least four pharmacologically distinct components: L-type current (NIM sensitive, 22%), N-type current (CgTx GVIA sensitive, 30%), Q-type current [Aga IVA (1 microM) and CgTx MVIIC sensitive, approximately 13-18%], and a resistant current.

OCULAR TOXICITY

Anand-Apte B, Pepper MS, Voest E, Montesano R, Olsen B, Murphy G, Apte SS, Zetter B. **Inhibition of angiogenesis by tissue inhibitor of metalloproteinase-3**. Invest Ophthalmol Vis Sci 1997;38(5):817-23.
PURPOSE: It has been established that Sorsby's fundus dystrophy, a dominantly inherited form of blindness, is caused by mutations in the tissue inhibitor of metalloproteinase-3 (TIMP-3) gene. Because choroidal neovascularization is a prominent feature of Sorsby's fundus dystrophy, the authors have examined whether TIMP-3 protein plays a role in the regulation of angiogenesis. METHODS: Chemotaxis of endothelial cells toward vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) was examined using a modified Boyden chamber assay. Endothelial cells placed in the upper chamber were allowed to migrate through a polycarbonate membrane with 8 microns pores toward VEGF or bFGF present in the lower chamber. Next, the ability of TIMP-3 to inhibit chemotaxis was studied by incubating the cells with varying amounts of TIMP-3 during the assay. Finally, an in vitro angiogenesis assay was performed on collagen gels. Endothelial cells were seeded onto three-dimensional collagen gels. Treatment with bFGF and VEGF induced invasion of the gel and the formation of tube-like structures. TIMPs (1, 2, and 3) were added to the cultures to determine their effect on invasion. An in vivo chorioallantoic membrane (CAM) assay was performed using methylcellulose discs containing bFGF with or without TIMP-3. Induction of new blood vessels was observed with a stereomicroscope. RESULTS: TIMP-3 inhibits chemotaxis of vascular endothelial cells toward VEGF and bFGF, inhibits collagen gel invasion and capillary morphogenesis in vitro, and inhibits bFGF-induced angiogenesis in the CAM assay in vivo. CONCLUSIONS: TIMP-3 has the potential to inhibit angiogenesis. These results allow us to speculate on a possible mechanism by which mutant TIMP-3 protein might contribute to the Sorsby fundus dystrophy phenotype.

Balls M, Botham PA, Bruner LH, Spielmann H. **The EC/HO international validation study on alternatives to the Draize eye irritation test**. Toxicol In Vitro 1995;9(6):871-929.
BIOSIS COPYRIGHT: BIOL ABS. This is the final report of the Management Team for a European Commission/ British Home Office (EC/HO) validation study on alternatives to the Draize eye irritation test. The principal goal of the study was to establish whether one or more of nine non-animal tests could be used to replace the Draize test for all severely irritating materials (or those belonging to specific classes) or the animal test completely for chemicals with or without regard to chemical class. Sixty chemicals were independently selected, coded and supplied, then the data obtained in 37 laboratories were analysed independently. The results of comparisons between 27 alternative test index scores and the Modified Maximum Average Scores (MMASs) obtained in the Draize eye test were compared. Tables of results showing Pearson's product moment correlation coefficients and Spearman's rank coefficients for each laboratory are provided, and correlation matrices of alternative test index scores among the different groups of laboratories are shown for each endpoint. Scatterplots are provided, in which the alternative test scores obtained by the lead laboratories for the nine tests are plotted against the MMAS for the full set of chemicals

and 12 surfactants. It is concluded that, with the possible exception of predicting the irritancy of surfactants, none of the nine tests met any of the four performance targets. Possible reasons for this outcome are discussed.

Barratt MD. **A quantitative structure-activity relationship for the eye irritation potential of neutral organic chemicals**. Toxicol Lett 1995;80(1-3):69-74.
Quantitative structure activity relationships (QSARs) were derived which related eye irritation data from a set of 46 neutral organic chemicals to the log octanol/water partition coefficient (logP), the minor principal inertial axes, and dipole moment. The data set consisted of four independent variables. It was analyzed by principal components analysis. The chemicals classified as eye irritants were clearly separated from those classified as nonirritants with the exception of isopropanol (67630) and methyl-acetate (79209). The selection of dipole moment as a parameter for the eye irritation QSAR was based on the knowledge that some low molecular weight neutral molecules can change the electrical resistance of phospholipid membranes. Of the 11 chemicals with conformation sensitive dipole moments, seven had conformations with dipoles aligned in their minimum energy confirmations, but for four of the chemicals, the conformation with the lowest energy resulted in their dipole being aligned antiparallel. The choice of logP together with parameters describing molecular dimensions was encouraged by conclusions of an earlier study indicating that eye irritation appeared dependent on log octanol/water partition coefficient and molecular size. The authors suggest that the characteristics of an eye irritant appear to be of a chemical which is hydrophobic enough to allow it to partition into a biological membrane while it also possesses a moderate dipole moment. The authors conclude that the QSARs as presented are expected to give reasonable predictions of the eye irritation potential of neutral organic chemicals and has the potential to be used initially as a prescreen prior to carrying out animal procedures for classifying new chemicals.

Botham P, Osborne R, Atkinson K, Carr G, Cottin M, Van Buskirk RG. **IRAG working group 3. Cell function-based assays. Interagency Regulatory Alternatives Group**. Food Chem Toxicol 1997;35(1):67-77.
Cell function-based tests measure responses of cells at sublytic concentrations of test agents. The fluorescein leakage assay measures effects of substances on the barrier function of epithelial monolayers or multilayers (MDCK or NHEK cells) as in vitro models of corneal epithelial function. Two IRAG data submissions suggest that the fluorescein leakage assay shows promise as a screening test for surfactants and alcohols. The test method requires further optimization, standardization and evaluation to fully determine its utility as an in vitro ocular.

Bradlaw J, Gupta K, Green S, Hill R, Wilcox N. **Practical application of non-whole animal alternatives: summary of IRAG workshop on eye irritation testing**. Interagency Regulatory Alternatives Group. Food Chem Toxicol 1997;35(1):175-8.
In November 1993, the Interagency Regulatory Alternatives Group (IRAG) sponsored a workshop to examine the current scientific status of alternatives to the Draize eye irritation test by assessing the current practical application of methods used to predict in vivo eye irritation. Laboratories from around the world were invited to submit detailed in vitro and in vivo data in parallel according to a specific set of guidelines in a consistent format. In vitro scores were compared with individual tissue scores. Over 60 data sets from 41 laboratories were received for 29 different test methods. Methods were grouped into five categories: organotypic models, chorioallantoic membrane-based assays, cell function-based assays, cytotoxicity assays and other systems. Data submissions and correlation analyses have been used to demonstrate the application of guidelines in method evaluations. Findings are summarized and future directions are indicated. A significant outcome of the workshop was the co-operation demonstrated among representatives of industry, academia and government in sharing test data on more than 2000 chemicals, products and product formulations for evaluation by their peers. Information obtained from this workshop will add to the weight of scientific evidence and scientific consensus about in vitro test methods and will establish credibility for regulatory acceptance of non-whole animal alternatives for ocular irritation.

Bruner LH, Spira H, Balls M, Hill RN. **Perspectives on alternatives to the eye irritation test: industry, public interest, government**. Food Chem Toxicol 1997;35(1):165-6.

Cassidy SL, Stanton E. **In vitro eye irritation studies on organosilicon compounds**. J Toxicol Cutan Ocular Toxicol 1997;16(1):45-60.
BIOSIS COPYRIGHT: BIOL ABS. The development of alternatives to in vivo eye irritation testing in animals is spurred by humane, scientific, and economic considerations. Two commercially available in vitro assays (Epi-Ocular Tissue Model OCL-100 and Skin2 ZK-1200 model) and a third published assay, the Bovine Corneal Opacity and Permeability Assay (BCOP), were used to assess the eye irritancy potential of several organosilicon (OS) compounds to evaluate their suitability for testing silicone polymers and other materials. The Skin2 ZK-1200 model (nine OS compounds) was generally applicable for the in vitro assessment of eye irritancy of liquid silicone polymers. However, some effort will be required to overcome the problem of working with viscous pastes and volatile low surface tension test materials for which variable results were observed. The correlation of the Skin2 model in vitro eye irritancy results with known in vivo data was generally good for silicone polymers, although some alkoxysilanes do not appear to give in vitro results that correspond with their in vivo irritancy. In vitro results obtained with the Epi-Ocular Tissue model OCL-100 (although limited) correctly identified both irritant and nonirritant silicone polymers. The BCOP assay correctly identified four nonirritant (two polydimethylsiloxanes, one phenylsilsesquioxane, and one silicone polyether) and two irritant (aminofunctional siloxane) polymers that are used extensively in personal care formulations. Histologic examination of the treated corneas yielded results that correlated well with the opacity and permeability endpoints. All three models performed well and are worthy of future study with OS compounds. Of the two tissue construct models, the Epi-Ocular Tissue model OCL-100 had several advantages over the Skin2 ZK-1200 model: it appeared to have a better technical design, facilitating more reliable dosing of the test materials and ease of use.

Chamberlain M, Gad SC, Gautheron P, Prinsen MK. **IRAG working group 1. Organotypic models for the assessment/prediction of ocular irritation**. Interagency Regulatory Alternatives Group. Food Chem Toxicol 1997;35(1):23-37.
The brief of the Organotypic Models Working Group was to review data submitted to the Interagency Regulatory Alternatives Group on the use of isolated eyes and components of the eye used to predict eye irritation potential. Data submissions were received on four test systems: the isolated rabbit eye (one submission), the isolated chicken eye (one submission), the bovine cornea (eight submissions) and the cultured bovine lens (one submission). On the basis of the data submitted on each test it was concluded that the isolated rabbit eye test as performed was capable of screening for severe eye irritants, but overall was of no practical value for determining irritation potential across the full range; that the isolated chicken eye test as performed showed promise as a method of predicting eye irritation potential, but the database was too small and needed expanding; that the bovine corneal opacity test had an extensive database and overall performed reasonably at screening out severe irritants and performed well for assigning relative potencies; and that the bovine lens test should be researched further to demonstrate its utility. The overall conclusion drawn was that the isolated eye tests and the bovine corneal opacity test can be used now to screen for severely irritating materials. However, it would be unwise to rely solely on these organotypic methods to provide evidence of lack of eye irritation hazard.

Cronin MT. **The use of cluster significance analysis to identify asymmetric QSAR data sets in toxicology. An example with eye irritation data**. SAR QSAR Environ Res 1996;5(3):167-75.
Cluster significance analysis is a tool that allows the identification of embedded clusters' in QSAR datasets. It is successfully applied to an eye irritation data set to show that these data are indeed asymmetric. The method identifies five parameters that form an embedded cluster of eye irritants amongst non irritants, although full separation is not achieved. This method has considerable potential to identify potential non-linearity in toxicology data sets and for parameter reduction. It is shown also that this can be obtained relatively quickly with an analysis performed on 100,000 subsets containing the same information as an analysis on 1,000,000 subsets.

Curren RD, Sina JF, Feder P, Kruszewski FH, Osborne R, Regnier JF. **IRAG working group 5. Other assays. Interagency Regulatory Alternatives Group**. Food Chem Toxicol 1997;35(1):127-58.
As part of the Interagency Regulatory Alternatives Group (IRAG) program to evaluate the state of the art in the development of alternative (non-whole animal) eye irritation tests, academic and industrial organizations were

invited to submit in vitro eye irritation data generated in their laboratories to one of several working groups for review. The assays reviewed in this report (from Working Group 5. Other Assays) were the EYTEX assay, tissue equivalent assay, a cytotoxicity assay using three-dimensional human fibroblast constructs, the Microtox assay, and other miscellaneous assays. Each submission consisted of raw data for chemicals and products tested, a description of the methodology, and an analysis (generally by regression analysis and Pearson's correlation coefficient) for the performance of the in vitro test relative to its ability to predict individual ocular tissue scores or total ocular score. In vivo data were generated according to the scoring methods proposed by Draize. Working Group 5 evaluated the submissions and commented on the utility of the assays. The variability of the in vivo data made conclusions difficult in many situations. Most of these assays were deemed useful (within limited chemical classes) for screening purposes or for use in conjunction with other toxicological information.

De Silva O, Cottin M, Dami N, Roguet R, Catroux P, Toufic A, Sicard C, Dossou KG, Gerner I, Schlede E, et al. **Evaluation of eye irritation potential: statistical analysis and tier testing strategies**. Food Chem Toxicol 1997;35(1):159-64.
Eye irritation testing, specifically the Draize test, has been the centre of controversy for many reasons. Several alternatives, based on the principles of reduction, refinement and replacement, have been proposed and are being used by the industry and government authorities. However, no universally applicable, validated non-animal alternative(s) is currently available. This report presents a statistical analysis and two testing approaches: the partial least squares multivariate statistical analysis of de Silva and colleagues from France, the tier-testing approach for regulatory purposes described by Gerner and colleagues from Germany, and the three-step tier-testing approach of the US Interagency Regulatory Alternatives Group described by Gupta and Hill. These approaches were presented as three separate papers at the November 1993 Interagency Regulatory Alternatives Group (IRAG) Workshop on Eye Irritation Testing; they have been summarized and combined into the following three-part report. The first part (de Silva et al.) presents statistical techniques for establishing test batteries of in vitro alternatives to the eye irritation test. The second (Gerner et al.) and third (Gupta and Hill) parts are similar in that they stage assessment of information by using a combination of screening information and animal testing to effect reductions in animal use and distress.

Fabrizio Saettone M, Chetoni P, Cerbai R, Mazzanti G, Braghiroli L. **Evaluation of ocular permeation enhancers: in vitro effects on corneal transport of four beta-blockers, and in vitro/in vivo toxic activity**. Int J Pharm 1996;142(1):103-13.
CBAC COPYRIGHT: CHEM ABS The efficacy and toxicity of a series of prospective ocular penetration enhancers (benzalkonium chloride, EDTA, nonionic surfactants, surface-active heteroglycosides and bile salts) was investigated in vitro, using isolated rabbit corneas. As test drugs 4 beta-blocking agents were used, chosen in order of increasing lipophilicity: atenolol (AT), timolol (TM), levobunolol (LB) and betaxolol (BX). The increased corneal hydration induced by the enhancers was taken as an index of cellular and tissue damage; the ocular irritancy of the agents was also tested in rabbits in vivo. In the absence of enhancers, the apparent corneal permeability coeffs. of the 4 drugs were in the order AT.simeq.TM

Feder P, Carr G, Holzhutter HG, Lovell D, Springer J. **Statistical planning and analysis considerations in the evaluation of in vitro alternatives to whole animal use for eye irritation testing**. Food Chem Toxicol 1997;35 (1):167-74.
Reports from the IRAG Working Groups assessing the current status of in vitro alternatives to whole animal eye irritation tests reflect some common approaches. Although each Working Group studied a particular class of assay, typically all Groups evaluated the in vitro alternatives on the basis of correlation with an in vivo test and the statistical significance of that correlation. However, the data furnished to them by the testing organizations had been obtained with little or no standardization of procedures for testing and evaluation of results. This paper presents issues of design and execution of such test programs that are of statistical concern, including objectives of the evaluation process; limitations of correlation; sources of variation and distinction between actual replication and repeated measurements; evaluation of the predictive ability of in vitro tests; association criteria; and other approaches to such evaluation programs. The distinction between statistical significance and toxicological

significance is pointed out. Suggestions are presented for standardization of test protocols.

Harbell JW, Koontz SW, Lewis RW, Lovell D, Acosta D. **IRAG working group 4. Cell cytotoxicity assays. Interagency Regulatory Alternatives Group**. Food Chem Toxicol 1997;35(1):79-126.
Twenty-seven data sets from 12 cellular cytotoxicity assays, intended to predict ocular irritation, were submitted to the Interagency Regulatory Alternatives Group (IRAG) for review. These data consisted of paired in vivo (Draize) and in vitro responses to individual chemicals and formulations. In vivo data consisted of individual tissue scores so that the predictive value of the in vitro assay could be assessed for each tissue response normally measured in the standard Draize assay. Data were compiled and evaluated according to the IRAG Guidelines Document. The Pearson's linear correlation coefficient was used as the first step in assessing the relationship between the in vitro and in vivo responses. The majority of the data sets represented the study of surfactant-based materials. In many cases, there was good correlation between the in vitro scores and the in vivo tissue responses. Most pronounced were the particularly good correlations between the in vitro scores and conjunctival redness scores across most of the assays. Based on the data submitted, a number of the cell cytotoxicity assays show considerable promise as screens for ocular irritancy. None of the submitters recommended that their cell cytotoxicity assay be used as a sole replacement for in vivo assessment. For almost all of these assays, the materials being tested should be water-soluble/miscible. The toxicity of products with reserve acidity or alkalinity or with high reactivity may be underestimated. A given user may prefer certain assays depending on the types of materials to be tested, the expected range of toxicities and the resources available. The cell cytotoxicity assays can serve as a valuable component of a tiered or battery testing program. As with any assay, a sufficient number of replicate values, concurrent positive and negative controls, and a strict adherence to assay acceptance criteria are essential to produce credible data.

Holzle E, Neumann N. **The photo hen's egg test: a novel model in phototoxicology. In: Holick MF, Jung EG, Editors. Biological Effects of Light 1995**. Proceedings of a Symposium, 4th; 1995 Oct 9-11; Atlanta, Ga. Berling: W. De Gruyter; 1996. P. 168-73
CBAC COPYRIGHT: CHEM ABS The aim of this investigation was to establish a new model for phototoxicity and photo-protection that is more advanced than the widely used cultures of yeasts, bacteria, or cells of various origin, at the same time avoiding animal testing. The authors studied the extraembryonal vasculature of the incubated hen's egg. This model was originally introduced by toxicologists as an alternative to the rabbit's eye irritation test, also known as Draize test. In the photo hem's egg test, test substances are applied to the embryo's yolk-sac blood vessel system which is then irradiated with 5 J/cm2 UVA (320-400 nm). The phototoxic agents promethazine, hematoporhyrin, ciprofloxacin, and 8-methoxypsoralen were tested in this system. To evaluate the photoprotective properties of ascorbic acid, acetylsalicylic acid, and indomethacin their effects on the phototoxic reaction induced by irradn. with 60 mJ/m2 UVB were studied. Death of the embryo, membrane discoloration, and hemorrhage were parameters for phototoxic damage, which were recorded during an observation period of 24 h. The phototoxic substances applied induced pronounced damage of the yolk-sac membrane and blood vessels which did not occur in the controls. These were test substances alone, UVA alone, or untreated eggs. The photoprotective agents were able to reduce significantly UVB induced damage to the yolk-sac membrane. The photo hen's egg test might be able to serve as a valid screening model for phototoxic of substances supposed to be photosensitizers and provides a model for evaluating photoprotective agents.

Kruszewski FH, Walker TL, Dipasquale LC. **Evaluation of a human corneal epithelial cell line as an in vitro model for assessing ocular irritation**. Fundam Appl Toxicol 1997;36(2):130-40.
CBAC COPYRIGHT: CHEM ABS A human corneal epithelial cell line, 10.014 pRSV-T (HCE-T cells), has been used to develop a three-dimensional in vitro model of the human corneal epithelium (HCE-T model). HCE-T cells form a stratified culture when grown at the air-liq. interface on a collagen membrane in serum-free medium. This model served as the basis for assays which supported the ocular irritancy assessment of water-sol. test substances. Cellular alterations in the HCE-T model were measured following 5-min topical exposures to 20 chems. [listed in the European Center for Ecotoxicol. and Toxicol. of Chems. (ECETOC) Ref. Chems. Data Bank] and 25 surfactant-based product formulations [utilized in the Cosmetic, Toiletry, and Fragrance Assocn. (CTFA)

Alternatives Program Phase III]. In vitro assays used were transepithelial permeability to sodium fluorescein (TEP) and transepithelial elec. resistance (TER). These measured alterations in the barrier function of this corneal epithelial equiv. Barrier function is a well-developed property in the HCE-T model that supports the mechanistic relevance of these assays. In vitro data, averaged from replicate assays, were compared to resp. Draize rabbit eye irritation data from the publicly available ECETOC and CTFA databases using linear regression with Pearson's correlation anal. For chems., Pearson's correlation coeffs., r, from comparisons of Draize max. av. scores (MAS) to TEP and TER data were 0.71 and 0.55, resp. For product formulations, Pearson's correlation coeffs. from comparisons of Draize MAS to TEP and TER data were 0.86 and 0.80, resp. Data indicated that barrier function alterations in the HCE-T model correlated with ocular irritancy and corneal toxicity. While the irritancy of the chems. tested was effectively assessed only by the TEP assay, that for the surfactant-based product formulations was effectively assessed by both the TEP and TER assays. Results also suggested that the HCE-T TEP and TER assays very in their effectiveness for evaluating specific classes of test materials.

Nourse WL, Tyson CA, Bednarz RM. **Mechanisms of mild ocular irritation**. Toxicol In Vitro 1995;9(6):967-76.
BIOSIS COPYRIGHT: BIOL ABS. Irritation and inflammation are complex processes. Several biochemical events are involved in the initiation, amplification and ultimate resolution of an inflammatory sequence, with the exact details depending on the nature of the insult. A wide variety of protocols have been proposed for the estimation of irritation potential, but none is fully accepted as a replacement for the Draize test. This selective review considers ideas generally neglected in toxicological research. Substantial support was found for the hypothesis that neurogenic phenomena play an important role in the irritant response, especially for the difficult case of mild irritation. The hypothesis is open to experimental evaluation by testing capsaicin-desensitized animals or those pretreated with recently developed pharmacological antagonists of neuropeptides. Fundamental interactions of perturbants with membranes and plasma proteins that are relevant to the initiation of the neurogenic response to mild irritation are discussed. Technical approaches for alternative assays based on the neurogenic hypothesis are considered.

Parnigotto PP, Bassani V, Gottardo A, Conconi MT, Valenti F. **Growth, morphology, morphometry and keratin patterns of bovine corneal epithelial cells cultured in vitro**. Anat Anz 1996;178(6):545-51.
In this study, the effects of different culture systems on bovine corneal epithelial cells were analysed in order to better understand the influence of bovine keratocytes on epithelial cells. Growth, morphological, morphometrical analyses of cells and keratin patterns were evaluated. The aim was to improve the culture technique in order to obtain a good in vitro proliferation of these cells for their employment in clinical and toxicological situations. The bovine corneal epithelial cells were cultured under different conditions: on keratocyte or 3T3-J2 fibroblast feeder layers, with media conditioned either by the two feeder layers or with a basal medium. The epithelial cells cultured on a keratocyte feeder layer as compared to those grown under the other conditions, proved to have a higher growth rate as well as to be smaller in the cytoplasmic and nuclear area; moreover, after 21 days of culture they expressed 64-kDa keratin, designed as a marker for corneal epithelial cell differentiation. To sum up, the keratocyte feeder layer is the most effective for stimulating the growth and differentiation of corneal epithelial cells, resembling the in vivo situation. It might also be successfully employed for clinical and toxicological purposes.

Scala RA, Springer J. **IRAG working group 6. Guidelines for the evaluation of eye irritation alternative tests: criteria for data submission. Interagency Regulatory Alternatives Group**. Food Chem Toxicol 1997;35(1):13-22.

Schmidt JF, Loeffler KU. **Toxicity and antiproliferative effect of aclacinomycin A on RPE cells in vitro**. Curr Eye Res 1996;15(11):1112-6.
PURPOSE: Aclacinomycin A or aclarubicin is an anthracycline that, by contrast with daunomycin, lacks carcinogenicity and is less toxic to the retina. We investigated the toxicity and antiproliferative effect of aclacinomycin A on retinal pigment epithelial cells that are known to play a mayor role in the pathogenesis of proliferative vitreoretinopathy. METHODS: In 3 experimental set-ups, RPE cells from pig eyes were incubated with

aclacinomycin A at different concentrations (0.5-15 micrograms/ml) and for various lengths of time (1-10 min). Cells were counted on day 3 after exposure to evaluate toxicity, subcultured, and counted once more on day 15 to test for the antiproliferative effect. Data were analyzed using the Tukey's Studentized Range (HSD) Test. Furthermore, RPE cells were examined by light microscopy. RESULTS: Cell numbers on day 3 after treatment were reduced significantly (p < OR = 0.05) Already at the lowest dosage tested (1 microgram/ML for 1 min). Higher doses, up to 15 Micrograms/ML for 5 min, did not lower cell numbers below 20% of those of control cultures. Logarithms of cell numbers on day 15 were inversely correlated to drug concentration as well as to incubation time. Cells that had been treated with 5 Micrograms/ML aclacinomycin A for 5 min were not able to start a new culture when subcultured 3 days after drug exposure. Conclusions: Aclacinomycin A applied intraocularly during vitreorentinal surgery may be an an alternative to daunomy in the treatment of proliferative vitreoretinopathy.

Spielmann H, Liebsch M, Moldenhauer F, Holzhutter HG, Bagley DM, Lipman JM, Pape WJ, Miltenburger H, De Silva O, Hofer H, et al. **IRAG working group 2. CAM-based assays. Interagency Regulatory Alternatives Group**. Food Chem Toxicol 1997; 35(1):39-66.
CAM-based assays, in which test material is applied to the chorion allantoic membrane (CAM) of embryonated chicken eggs, were assessed as alternatives to the Draize eye irritation test. Two general types of CAM-based assays are currently in use, the HET-CAM test and the CAMVA assay. Evaluations were made of five data sets produced with three different modifications of the HET-CAM test and two data sets obtained with the same CAMVA protocol. Data sets consisted of 9-133 test chemicals, usually from the sponsor's product line, and also from a validation trial. Each data set and assay protocol were analysed for quality of data, purpose and proposed use of the assay, range of responses covered, range of test materials amenable, current use in safety and risk assessment both in-house and for regulatory purposes. Since the MMAS Draize score was not available for all in vivo data sets, the sigma MMMIS, which correlates well with the MMAS, was used instead. In vitro/in vivo correlations calculated with Pearson's linear coefficient ranged from r = 0.6 to r = 0.9 for six of seven data sets. Corneal opacity and inflammation of the iris showed the best correlation to in vitro data. Prediction rates were significantly improved when partial linear regression was used, and the predictivity of three different HET-CAM protocols was almost the same. HET-CAM assays showed the best prediction with surfactants and surfactant-based formulations, whereas the CAMVA assay provided the best performance with alcohols.

PHARMACOKINETIC AND MECHANISTIC STUDIES

Abu-Izza K, Tambrallo L, Lu DR. **In vivo evaluation of zidovudine (AZT)-loaded ethyl cellulose microspheres after oral administration in beagle dogs**. J Pharm Sci 1997;86(5):554-9.
CBAC COPYRIGHT: CHEM ABS The purpose of this study was to evaluate the in vivo performance of sustained-release zidovudine (AZT) microspheres after oral administration in beagle dogs, and to establish an in vitro-in vivo correlation. Two AZT microsphere formulations as well as AZT powder were administered to 4 Beagle dogs. The plasma concn.-time data was analyzed by both compartmental and noncompartmental pharmacokinetic analyses. Based on the calcd. pharmacokinetic parameters, in vivo release profiles were simulated and compared with in vitro release profiles in 3 different release media. Significantly longer mean residence time (MRT) was obsd. after administration of the sustained-release microspheres compared with AZT powder. Significantly lower max. (Cmax) concn. values and longer times to Cmax (tmax) values were also obsd. Formulation I showed the longest MRT (4.4 h). AZT plasma concn. was maintained above the min. effective concn. for .apprx.10 h after administration of Formulation I. The relative bioavailability of the microsphere formulations with respect to AZT powder was not significantly different from 1. The in vitro release of the 3 formulations was slower in simulated gastric fluid compared with simulated intestinal fluid. The addn. of enzymes and mucin to the release media significantly lowered the in vitro release rate of AZT from the microspheres formulations, but not from AZT powder. A good level of in vitro-in vivo correlation (Level A correlation) was achieved with a release medium that was composed of simulated gastric fluid with pepsin and mucin for 2 h followed by simulated intestinal fluid with pancreatin and mucin

for 8 h. This in vitro model may be used to predict the in vivo release of AZT, in the further development of controlled-release AZT formulations.

Adams PC, Rickert DE. **The absorption and first-pass metabolism of [14C]-1,3-dinitrobenzene in the isolated vascularly perfused rat small intestine**. Biopharm Drug Dispos 1996;17(8):675-98.
We tested the hypothesis that the small intestine is capable of the first-pass, reductive metabolism of xenobiotics. A simplified version of the isolated vascularly perfused rat small intestine was developed to test this hypothesis with 1,3-dinitrobenzene (1,3-DNB) as a model xenobiotic. Both 3-nitroaniline (3-NA) and 3-nitroacetanilide (3-NAA) were formed and absorbed following intralumenal doses of 1,3-DNB (1.8 or 4.2 mumol) to isolated vascularly perfused rat small intestine. Dose, fasting, or antibiotic pretreatment had no effect on the absorption and metabolism of 1,3-DNB in this model system. The failure of antibiotic pretreatment to alter the metabolism of 1,3-DNA indicated that 1,3-DNB metabolism was mammalian rather than microfloral in origin. All data from experiments initiated with lumenal 1,3-DNB were fit to a pharmacokinetic model (model A). ANOVA analysis revealed that dose, fasting, or antibiotic pretreatment had no statistically significant effect on the model-dependent parameters. 3-NA (1.5 mumol) was administered to the lumen of isolated vascularly perfused rat small intestine to evaluate model A predictions for the absorption and metabolism of this metabolite. All data from experiments initiated with 3-NA were fit to a pharmacokinetic model (model B). Comparison of corresponding model-dependent pharmacokinetic parameters (i. e. those parameters which describe the same processes in models A and B) revealed quantitative differences. Evidence for significant quantitative differences in the pharmacokinetics or metabolism of formed versus preformed 3-NA in rat small intestine may require better definition of the rate constants used to describe tissue and lumenal processes or identification and incorporation of the remaining unidentified metabolites into the models.

Akamatsu M, Ozoe Y, Ueno T, Fujita T, Mochida K, Nakamura T, Matsumura F. **Sites of action of noncompetitive GABA antagonists in houseflies and rats: three-dimensional QSAR analysis**. Pestic Sci 1997;49(4):319-32.
CBAC COPYRIGHT: CHEM ABS Quant. structure-activity relationships for insecticidal activity (against houseflies) and competitive activity against a specific [35S]tert-butyl-bicyclophosphorothionate binding (to rat brain membranes) of some picrotoxinin-type 4-aminobutyric acid antagonists, including gamma-BHC, endosulfan, bicyclophosphates, dioxatricyclododecenes and related compds., were examd. three-dimensionally using comparative mol. field anal. (CoMFA). The antagonists were classified into two series according to their mol. shapes: i.e. whether their structure was 'linearly' extended beyond the 'mast-head' position of the 'boat-like' skeletons (series 1) or not (series 2). CoMFA showed that the slopes in steric and electrostatic fields around the mol. were significant for both series in governing the potency variations in insecticidal and binding activities. Hydrophobicity, a possible factor controlling transport behavior of compds., was significant in governing variations in insecticidal activity, but not for the case of the rat membrane binding. Assuming that there is a slight topol. difference between series 1 and 2 compds. in terms of the mode of binding with the housefly receptor site, the insecticidal activity was analyzable with a single equation for the combined set of compds., but the rat membrane binding was not. The sterically and electrostatically favorable regions surrounding the mol. series indicated by CoMFA were roughly located at positions so as to interact with the binding subsites on the receptors proposed previously.

Andersen ME, Birnbaum LS, Barton HA, Eklund CR. **Regional hepatic CYP1A1 and CYP1A2 induction with 2,3,7,8-tetrachlorodibenzo-p-dioxin evaluated with a multi-compartment geometric model of hepatic zonation**. Toxicol Appl Pharmacol 1997;144(1):145-55.
CBAC COPYRIGHT: CHEM ABS A physiol. based pharmacokinetic (PBPK) model for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was combined with a five-compartment geometric model of hepatic zonation to predict both total and regional induction of CYP450 proteins within the liver. Three literature studies on TCDD pharmacokinetics and protein induction in female rats were analyzed. In simulating low-dose behavior for mRNA in whole liver and, particularly, in representing immunohistochem. observations, the five-compartment model was more successful than conventional homogeneous one-compartment liver models. The five-compartment liver model was used with the affinity of TCDD for the Ah receptor (AhR) held const. across all the liver (Kb = 0.2 nM). The presumed affinities of the AhR-TCDD complex for TCDD responsive elements in the CYP1A1 (Kd1) and CYP1A2 (Kd2) genes varied between adjacent compartments by a factor of 3. This parameterization leads to predicted 81-fold differences in

affinities between the centrilobular and the periportal regions. The affinities used for AhR-TCDD complex binding to TCDD response elements for CYP1A2 in compartment 3 (the midzonal area) ranged from 0.08 to 1.0 nM in the three studies modeled. For CYP1A1 the corresponding dissocn. const. in compartment 3 varied from 0.6 to 2.0 nM. In each compartment, the Hill coeff. for induction had to be 4 or greater to match the immunohistochem. results. This multi-compartment liver model is consistent with data on protein and mRNA induction throughout the liver and on the regional distribution of these proteins. No previous model has incorporated regional variations in induction. The PBPK anal. based on the multi-compartment liver model suggests that the low-dose behavior for hepatic CYP1A1/CYP1A2 induction by TCDD is highly nonlinear.

Artalejo CR, Lemmon MA, Schlessinger J, Palfrey HC. **Specific role for the PH domain of dynamin-1 in the regulation of rapid endocytosis in adrenal chromaffin cells**. Embo J 1997;16(7):1565-74.
Dynamin plays a key role in the scission event common to various types of endocytosis. We demonstrate that the pleckstrin homology (PH) domain of dynamin-1 is critical in the process of rapid endocytosis (RE) in chromaffin cells. Introduction of this isolated PH domain into cells at concentrations as low as 1 microM completely suppressed RE. PH domains from other proteins, including that from the closely related dynamin-2, were ineffective as inhibitors, even at high concentrations. Mutational studies indicated that a pair of isoform-specific amino acids, located in a variable loop between the first two beta-strands, accounted for the differential effect of the two dynamin PH domains. Switching these amino acids in the dynamin-2 PH domain to the equivalent residues in dynamin-1 (SL-->GI) generated a molecule that blocked RE. Thus, the PH domain of dynamin-1 is essential for RE and exhibits a precise molecular selectivity. As chromaffin cells express both dynamin-1 and -2, we speculate that different isoforms of dynamin may regulate distinct endocytotic processes and that the PH domain contributes to this specificity.

Audet R, Rioux F, Drapeau G, Marceau F. **Cardiovascular effects of Sar-(D-Phe8)des-Arg9-bradykinin, a metabolically protected agonist of B1 receptor for kinins, in the anesthetized rabbit pretreated with a sublethal dose of bacterial lipopolysaccharide**. J Pharmacol Exp Ther 1997;280(1):6-15.
BIOSIS COPYRIGHT: BIOL ABS. We investigated the mechanism of the hypotensive effect of Sar-(D-Phe8)des-Arg9-bradykinin (BK) in lipopolysaccharide-treated anesthetized rabbits. The study involved pharmacokinetic and hemodynamic measurements and tests of antagonism with various drugs. The rate of elimination of Sar-(D-Phe8) des-Arg9-BK from the rabbit plasma was slower than that of Lys-BK, a naturally occurring B1 agonist. The amplitude of the hypotensive effect of Sar-(D-Phe8)des-Arg9-BK was not affected by pretreatment with indomethacin, diclofenac, dazmegrel, NG-nitro-L-arginine, glibenclamide, MK-886, BN-50739, atropine or propranolol, but its duration was shortened by indomethacin and diclofenac. Sar-(D-Phe8)des-Arg9-BK-induced hypotension was associated with decreases of total peripheral resistance, cardiac output, carotid, mesenteric and femoral blood flow, transient reductions followed by secondary increases of vascular resistance in the carotid and femoral beds, reductions of central venous pressure, but no change of hematocrit. Animal pretreatment with diclofenac or hexamethonium abolished the secondary increases of carotid bed vascular resistance caused by the B1 agonist. These and other results suggest that peripheral vasodilation leading to a decrease of total peripheral resistance and a decrease of cardiac output may both contribute consecutively to the hypotensive effect of Sar-(D-Phe8)des-Arg9-BK in this animal model. Inappropriate compensatory responses to arterial hypotension, prostaglandin release, and slow rate of elimination of Sar-(D-Phe8)des-Arg9-BK from the rabbit plasma, may all be at the basis of the prolonged duration of the hypotension caused by the B1 agonist.

Bachurin SO, Shevtzova EP, Lermontova NN, Serkova TP, Ramsay RR. **The effect of dithiocarbamates on neurotoxic action of 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) and on mitochondrial respiration chain**. Neurotoxicology 1996;17(3-4):897-903.
BIOSIS COPYRIGHT: BIOL ABS. The neurotoxin MPTP induces in human and in some laboratory animals parkinsonism-like neurological disorder, biochemically characterized by selective and irreversible decrease of dopamine content in striatum. The terminal step in the mechanism of neurotoxic action of MPTP is the inhibition of mitochondrial respiratory chain by pyridinium metabolite (MPP+) resulting in energy depletion and nervous cells death. Earlier it was shown that some chemical compounds, in particular diethyldithiocarbamate (DTC), can

potentiate MPTP neurotoxicity. In the present work we have studied the influence of DTC derivatives on MPTP neurotoxic effect in vivo and on MPP+ inhibition of mitochondrial respiration (both on intact mitochondria and on submitochondrial particles) in vitro. It was revealed that DTC alone change mitochondrial membrane state by respiratory chain uncoupling and inhibition. DTC and MPP+ mutually potentiate inhibition of electron transport as well. The combined effect of DTC plus MPP+ action on mitochondria respiration reflects the sum of reciprocally leveling and potentiating factors and can explain the order of efficacy of MPTP-neurotoxicity potentiation in vivo in series of close DTC derivatives.

Bois FY, Gelman A, Jiang J, Maszle DR, Zeise L, Alexeef G. **Population toxicokinetics of tetrachloroethylene**. Arch Toxicol 1996;70(6):347-55.
A pharmacokinetic model, based on Markov chain Monte Carlo simulation, was used to predict the fraction of tetrachloroethylene (127184) (PERC) metabolized in humans. A previously described four compartment model was chosen to simulate PERC metabolism. Published data on six male subjects exposed to 72 and 144 parts per million PERC, served as data input for the model. A hierarchical population model with an individual and a population level was employed. Predicted values were derived as a function of exposure level, time, a set of unknown physiological parameters, and a set of measured, covariant parameters. A close fit of the predicted values with the observed data was obtained using the model. According to the model, the mean fraction of PERC metabolized at low exposure, ambient inhalation levels, was 36% with a standard deviation of 11%. The corresponding mean value for high exposure, exceeding occupational standards, was 1.7% with a deviation of 0.95%. The 95% confidence intervals were 15% and 0.52% for low and high exposures, respectively. The authors conclude that the higher fraction of PERC metabolized by humans at lower levels of exposure should be taken into account when setting safe exposure limits for PERC.

Bouzyk E, Iwanenko T, Jarocewicz N, Kruszewski M, Sochanowicz B, Szumiel I. **Antioxidant defense system in differentially hydrogen peroxide sensitive L5178Y sublines**. Free Radic Biol Med 1997;22(4):697-704.
Two sublines of L5178Y (LY) murine lymphoma, differing in sensitivity to hydrogen peroxide, served as a cellular model for examination of the antioxidant defense system. The contribution of catalase, glutathione peroxidase (G-Px) and glutathione were evaluated. Sensitivity to 3-amino-1,2,4-triazole (AMT), inhibitor of catalase, was higher in LY-R (hydrogen peroxide sensitive) than in LY-S (hydrogen peroxide resistant) cells. Accordingly, activity of catalase was twofold lower in LY-R than in LY-S cells. G-Px activity was about two times higher in LY-R than in LY-S cells. After induction with selenium it increased 15.6 times in LY-R cells and 50.3 times in LY-S cells. Reduced glutathione (GSH) content (and possibly other monobromobimane-reactive thiols) were determined fluorimetrically with monobromobimane and fluorescence found 54% higher in LY-S than in LY-R cells. Inhibition of catalase caused GSH decrease in LY-S cells; this decrease was abrogated by inducing G-Px by selenium treatment. On the contrary, in LY-R cells inhibition of catalase decreased GSH content only slightly and selenium treatment did not further change the GSH level. DNA damage (estimated by comet assay) was the same in hydrogen peroxide-treated cells in the presence or absence of AMT; however, after induction of G-Px by selenium, DNA damage was considerably lowered. This sparing effect of selenium was accompanied by decreased growth inhibition in selenium pretreated, hydrogen peroxide-treated cell cultures.

Capen CC. **Mechanistic data and risk assessment of selected toxic end points of the thyroid gland**. Toxicol Pathol 1997;25(1):39-48.
Many goitrogenic xenobiotics that increase the incidence of thyroid tumors in rodents exert a direct effect on the thyroid gland to disrupt one of several possible steps in the biosynthesis, secretion, and metabolism of thyroid hormones. This includes (a) inhibition of the iodine trapping mechanism, (b) blockage of organic binding of iodine and coupling of iodothyronines to form thyroxine (T4) and triiodothyronine (T3), and (c) inhibition of thyroid hormone secretion by an effect on proteolysis of active hormone from the colloid. Another large group of goitrogenic chemicals disrupts thyroid hormone economy by increasing the peripheral metabolism of thyroid hormones through an induction of hepatic microsomal enzymes. This group includes central nervous system-acting drugs, calcium channel blockers, steroids, retinoids, chlorinated hydrocarbons, polyhalogenated biphenyls, and enzyme inducers. Thyroid hormone economy also can be disrupted by xenobiotics that inhibit the 5'-monodeiodinase that converts T4

in peripheral sites to biologically active T3. Inhibition of this enzyme by FD&C Red No. 3 lowers circulating T3 levels, which results in a compensatory increased secretion of thyroid-stimulating hormone (TSH), follicular cell hypertrophy and hyperplasia, and an increased incidence of follicular cell tumors in 2-yr or lifetime studies in rats. Physiologic perturbations alone, such as the feeding of an iodine-deficient diet, partial thyroidectomy, natural goitrogens in certain foods, and transplantation of TSH-secreting pituitary tumors in rodents also can disrupt thyroid hormone economy and, if sustained, increase the development of thyroid tumors in rats. A consistent finding with all of these goitrogens, be they either physiologic perturbations or xenobiotics, is the chronic hypersecretion of TSH, which places the rodent thyroid gland at greater risk to develop tumors through a secondary (indirect) mechanism of thyroid oncogenesis associated with hormonal imbalances.

Carlier R, Raoult E, Tallec A, Andre V, Gauduchon P, Lancelot JC. **Electrochemical behavior of mutagenic nitro and amino derivatives of carbazole**. Electroanalysis 1997;9(1):79-86.
BIOSIS COPYRIGHT: BIOL ABS. The electrochemical behavior of nitro and amino carbazole derivatives has been investigated. In aprotic medium, both in reduction and oxidation, the electrochemical process is complicated by father-son protonation reactions. In protic medium, the hydroxylamines resulting from 4-electron reduction of nitrocarbazoles are unstable in very acidic medium, but relatively stable in acetic or ammoniacal buffer, except for 3-hydroxylaminocarbazole; reduction of amino-nitrocarbazoles leads to unstable hydroxylamines. Oxidation of aminocarbazoles is a 2-electron process, the 2-amino compounds being more difficult to oxidize than the 3-amino derivatives. The relationships between these electrochemical behaviors and mutagenic properties of the studied compounds are also discussed.

Chiarpotto E, Biasi F, Scavazza A, Camandola S, Aragno M, Tamagno E, Danni O, Dianzani MU, Poli G. **Acetaldehyde involvement in ethanol-induced potentiation of rat hepatocyte damage due to the carcinogen 1,2-dibromoethane**. Alcohol Alcohol 1995;30(6):721-8.
BIOSIS COPYRIGHT: BIOL ABS. Previous experiments with hepatocytes isolated from ethanol-treated rats showed that alcohol potentiates the toxic action of 1.2-dibromoethane (DBE) by inhibiting its metabolism via glutathione-S-transferase. The aim of this study was to investigate whether acetaldehyde, the main product of ethanol metabolism, may be responsible for such inactivation. By pretreatment with 4- methylpyrazole, an inhibitor of acetaldehyde formation, the ethanol inactivation of glutathione transferase was actually prevented. As a consequence of this protective action, 4-methylpyrazole also prevented the high basal lipid peroxidation and the potentiated DBE toxicity observed in hepatocytes from ethanol-dosed animals. Finally, the inactivation of glutathione-S-transferase by concentrations of acetaldehyde likely to occur in the ethanol-intoxicated animal was confirmed in an in vitro model by direct aldehyde addition to hepatocyte suspensions.

Clewell HJ, 3d . **The application of physiologically based pharmacokinetic modeling in human health risk assessment of hazardous substances**. Toxicol Lett 1995;79(1-3):207-17.
The applications of physiologically based pharmacokinetic (PBPK) modeling and its capabilities, challenges, and limitations were reviewed. PBPK modeling attempts to describe the relationship between external measures of applied dose and internal measures of biologically active dose, using a realistic description of mammalian physiology and biochemistry. Nonlinear biological processes can be added and the model supports cross species extrapolation. The key capability of PBPK modeling is dealing with interindividual variation in the human population. PBPK modeling can identify physiological and biochemical parameters that determine individual risk. The key challenge for PBPK modeling is evaluating model adequacy as to whether predictions are accurate. This includes specifying model parameters, validating that the model predicts the behavior of the chemical under conditions that test the underlying mechanistic structure, and modifying the model. The key limitation of PBPK modeling is obtaining the necessary experimental data. Physiological parameters and partition coefficients are usually available in the literature, but metabolism parameters are the most difficult to obtain. The author concludes that applications of PBPK modeling in health risk assessment range from very simple to very complex. As more advanced chemical specific applications expand, PBPK modeling will be applied to less studied environmental contaminants.

Cohen SD, Pumford NR, Khairallah EA, Boekelheide K, Pohl LR, Amouzadeh HR, Hinson JA. **Selective protein covalent binding and target organ toxicity**. Toxicol Appl Pharmacol 1997;143(1):1-12.
Protein covalent binding by xenobiotic metabolites has long been associated with target organ toxicity but mechanistic involvement of such binding has not been widely demonstrated. Modern biochemical, molecular, and immunochemical approaches have facilitated identification of specific protein targets of xenobiotic covalent binding. Such studies have revealed that protein covalent binding is not random, but rather selective with respect to the proteins targeted. Selective binding to specific cellular target proteins may better correlate with toxicity than total protein covalent binding. Current research is directed at characterizing and identifying the targeted proteins and clarifying the effect of such binding on their structure, function, and potential roles in target organ toxicity. The approaches employed to detect and identify the tartgeted proteins are described. Metabolites of acetaminophen, halothane, and 2,5-hexanedione form covalently bound adducts to recently identified protein targets. The selective binding may influence homeostatic or other cellular responses which in turn contribute to drug toxicity, hypersensitivity, or autoimmunity.

Dees C, Askari M, Henley D. **Carcinogenic potential of benzene and toluene when evaluated using cyclin-dependent kinase activation and p53-DNA binding**. Environ Health Perspect 1996;104(Suppl 6):1289-92.
Benzene is carcinogenic, whereas toluene is thought to have little carcinogenic potential. Benzene and toluene were found to activate cyclin-dependent kinase 2 in rat liver epithelial (RLE) and HL60 cells. pRb105 was hyperphosphorylated in RLE cells treated with either solvent. Kinase activation and subsequent hyperphosphorylation of pRb105 and p53 by benzene or toluene may be responsible for their growth promotional effects, but it does not account for increased potential of benzene to induce cancer. Therefore, we examined the ability of these solvents to increase p53-DNA site-specific binding in RLE cells. Benzene increased p53-DNA site-specific DNA binding in RLE cells compared to control levels or the effects of toluene. Increased p53-DNA site-specific binding by benzene may be caused by damage to cellular DNA. If so, although both solvents appear to have promotional activity, the increased potential of benzene to damage DNA may be responsible to the difference in the ability of benzene to cause cancer.

Di Fabio R, Capelli AM, Conti N, Cugola A, Donati D, Feriani A, Gastaldi P, Gaviraghi G, Hewkin CT, Micheli F, et al. **Substituted indole-2-carboxylates as in vivo potent antagonists acting as the strychnine-insensitive glycine binding site**. J Med Chem 1997;40(6):841-50.
A series of indole-2-carboxylates bearing suitable chains at the C-3 position of the indole nucleus was synthesized and evaluated in terms of in vitro affinity using [3H]glycine binding assay and in vivo potency by inhibition of convulsions induced by N-methyl-D-aspartate (NMDA) in mice. 3-[2-[(Phenylamino)carbonyl]ethenyl]-4,6-dichloroindole-2-carboxy- l ic acid (8) was an antagonist at the strychnine-insensitive glycine binding site (noncompetitive inhibition of the binding of [3H]TCP, pA2 = 8.1) displaying nanomolar affinity for the glycine binding site (pKi = 8.5), coupled with high glutamate receptor selectivity (> 1000-fold relative to the affinity at the NMDA, AMPA, and kainate binding sites). This indole derivative inhibited convulsions induced by NMDA in mice, when administered by both iv and po routes (ED50 = 0.06 and 6 mg/kg, respectively). The effect of the substituents on the terminal phenyl ring of the C-3 side chain was investigated. QSAR analysis suggested that the pKi value decreases with lipophilicity and steric bulk of substituents and increases with the electron donor resonance effect of the groups present in the para position of the terminal phenyl ring. According to these results the terminal phenyl ring of the C-3 side chain should lie in a nonhydrophobic pocket of limited size, refining the proposed pharmacophore model of the glycine binding site associated with the NMDA receptor.

Ette EI, Kelman AW, Howie CA, Whiting B. **Analysis of animal pharmacokinetic data: performance of the one point per animal design**. J Pharmacokinet Biopharm 1995 Dec;23:551-66.
IPA COPYRIGHT: ASHP A simulation study to determine the impact of various design factors on the accuracy and precision with which population pharmacokinetic parameters are estimated in preclinical studies was conducted for a drug given by intravenous injection and having monoexponential disposition characteristics. The one observation per animal design yielded biased and imprecise estimates of variability, and residual variability could not be estimated. Increasing the error in concentration measurement led to deterioration in the accuracy and precision with

which variability was estimated. Obtaining a second sample from each animal practically eliminated bias and facilitated partitioning of interanimal variability and residual intraanimal variability. Doubling the total number of observations per animal required using half the total number of animals required for accurate and precise parameter estimation with the one sample per animal design.

Fernandez N, Sierra M, Diez MJ, Teran T, Pereda P, Garcia JJ. **Study of the pharmacokinetic interaction between ethinylestradiol and amoxicillin in rabbits**. Contraception 1997;55(1):47-52.
Several antibiotics have been implicated in oral contraception failure when they are administered at the same time as the oral contraceptive (OC) pill. In the present paper, a study about amoxicillin-ethinylestradiol (EE2) pharmacokinetic potential interaction was studied. Two rabbit groups were utilized, the first group received amoxicillin (10 mg/kg) and EE2 (30, 50 and 100 micrograms/kg, respectively), both by intravenous (i.v.) route. The second group received amoxicillin (oral route, 10 mg/kg/day) and EE2 (i.v. route, 100 mu/kg) on day 1, 4 and 8 of antibiotic treatment, respectively. After compartmental (two-compartment open model) and non-compartmental analysis of plasma concentrations, the statistical study (ANOVA p < OR = 0.05) revealed that the presence of amoxicillin did not modify the EE2 distribution and elimination pharmakokinetic parameters (by comparison with those obtained in a previous study where EE2 was administered alone.) There also were no significant differences with the time of Amolxicillin oral treatment.

Figg WD, Pluda JM, Lush RM, Saville MW, Wyvill K, Reed E, Yarchoan R. **The pharmacokinetics of TNP-470, a new angiogenesis inhibitor.** Pharmacotherapy 1997;17(1):91-7.
CBAC COPYRIGHT: CHEM ABS To characterize the pharmacokinetic profile of TNP-470, a synthetic analog of fumagillin that is a potent inhibitor of angiogenesis and inhibits neovascularization in several solid tumor models. The TNP-470 dosage was increased in 13 sequential cohorts using a modified Fibonacci escalation scheme (4.6, 9.3, 15.4, 23.2, and 43.1 mg/M2). The drug was administered as a 1-h i.v. infusion. Serial blood samples were collected and assayed by reverse-phase high-performance liq. chromatog. and the pharmacokinetics were characterized. There was a linear relationship between the dose of TNP-470 and both area under the curve to infinity (AUC[inf]) and time to max. concn. (Cmax). The Cmax ranged between 6.6 ng/mL at the lowest dosage (4.6 mg/M2) and 597.1 ng/mL at the highest dosage (43.1 mg/M2). The agent was rapidly cleared from the circulation with a short terminal half-life (0.88.+-.2.5 h), which is consistent with preclin. data. Peak plasma concns. of AGM-1883, an active metabolite, ranged between 0.4 and 158.1 ng/mL. Concns. of TNP-470 that have in vitro activity were achievable in vivo. The drug was rapidly cleared from the circulation after a single 1-h infusion. There was considerable interpatient variability in the clearance, but no evidence of saturable elimination. If more prolonged exposure is necessary for activity, administration of TNP-470 by continuous infusion may be suitable.

Garin MI, Lopez RM, Luque J. **Pharmacokinetic properties and in-vivo biological activity of recombinant human erythropoietin encapsulated in red blood cells**. Cytokine 1997;9(1):66-71.
CBAC COPYRIGHT: CHEM ABS The in-vivo survival of 51Cr-labeled murine red blood cells (RBCs) loaded with recombinant human erythropoietin (rhEpo-RBCs) was slightly lower than that of normal RBCs. I.v. administration to normal mice of the encapsulated rhEpo shows the pharmacokinetic bicompartmental profile typical of the free rhEpo. Distribution and elimination half-life values for the RBC-entrapped rhEpo were no longer than those for the free protein. The area under the curve value was significantly increased for rhEpo-RBCs. Hypertransfused polycythemic mice were evaluated as an adequate animal model to study the in vivo biol. activity of encapsulated rhEpo. RhEpo-RBCs stimulate the erythropoiesis of polycythemic mice in a linear dose-radio-iron incorporation response relationship. These results suggest that rhEpo-RBCs may behave as an alternative to the administration of free rhEpo in the clin. field.

Giardino NJ. **An indoor air quality-pharmacokinetic simulation of passive inhalation of marijuana smoke and the resultant buildup of 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid in urine**. J Forensic Sci 1997;42(2):323-5.
In military courts of law, the good soldier defense is often used by the defendant to explain the presence of 11-nor-

delta-9-tetrahydrocannabinol-9-carboxylic acid in urine (hereafter referred to as THCA) above the Department of Defense (DOD) established limit of 15 ng/mL. The defense will contend the defendant unwittingly breathed side-stream marijuana smoke, thus resulting in the presence of THCA in the defendant's urine. The purpose of this work was to link an indoor air quality model (IAQ) with a pharmacokinetic (PK) model to predict a passive marijuana smoker's resultant concentration of the major urinary metabolite THCA.

Green T. **Methylene chloride induced mouse liver and lung tumours: an overview of the role of mechanistic studies in human safety assessment**. Hum Exp Toxicol 1997;16(1):3-13.
BIOSIS COPYRIGHT: BIOL ABS. B6C3F1 mice exposed to high dose levels of methylene chloride by inhalation for 2 years had an elevated incidence of liver and lung tumours. These tumours were not increased in rats or hamsters exposed under the same or similar conditions. This paper gives an overview of research conducted over the last 10 years into the mechanism of action of methylene chloride as a mouse carcinogen and into the relevance of the mouse data to humans exposed to this chemical. Data are presented on the comparative metabolism and pharmacokinetics of methylene chloride in mice, rats, hamsters and humans, on the toxicity of methylene chloride to the target organs in the mouse, and on the genotoxicity of methylene chloride in vitro and in vivo. The enzyme which activates methylene chloride to its carcinogenic form has been isolated, sequenced, and cloned, and its distribution studied within cells, organs and between species. Evidence has been obtained to show that methylene chloride caused cancer in mice as a result of interactions between metabolites of the glutathione S-transferase pathway and DNA. Damage to mouse lung Clara cells and increased cell division are believed to have influenced the development of the lung tumours. The species specificity was a direct consequence of the very high activity and specific cellular and nuclear localisation of a thieta class glutathione S-transferase enzyme which was unique to the mouse. Consequently, DNA damage was not detectable in rats in vivo, or in hamster and human hepatocytes exposed to cytotoxic dose levels of methylene chloride in vitro. These results provide evidence that the mouse is unique in its response to methylene chloride and that it is an inappropriate model for human health assessment.

Hata K, Komoriya K, Takada N, Kobayashi H, Ohnuma N, Hayashi Y. [**Pharmacokinetic studies of lanoteplase, a novel tissue plasminogen activator (1). Plasma levels after a single bolus intravenous administration in rats, rabbits and dogs**]. Oyo Yakuri 1997;53(1):1-10. (Jpn)
CBAC COPYRIGHT: CHEM ABS Lanoteplase is a novel recombinant human tissue plasminogen activator deleted the finger and growth factor domains and modified glycosylation site at the 117 amino acid residue of human tissue plasminogen activator (t-PA). The pharmacokinetics of lanoteplase was investigated in rats, rabbits and dogs in comparison with those of alteplase, a recombinant human t-PA, after a single bolus injection. In rats, the elimination half-lives of lanoteplase at the dose of 0.025, 0.1 and 1.0 mg/kg were 9-11 min for alpha-phase and 64-70 min for beta-phase, resp., whereas those of alteplase at the same doses were 2.1-2.7 min for alpha-phase and 25-44 min for beta-phase, resp. Lanoteplase had a longer half-life than t-PA in rats. The distribution vol. of central compartment (V1) and total body clearance (CLtot) of lanoteplase were 26-37 mL/kg and 0.62-0.72 mL/min/kg, resp., showing linearity in the pharmacokinetics. The AUC values were 16 times more than that of t-PA and CLt0t values, were 1/16 times less than that of t-PA. Rat models of hepatic and renal insufficiency achieved by vascular ligations were used to evaluate the acute effects on pharmacokinetics of lanoteplase with or without clearance region for t-PA. The pharmacokinetics of lanoteplase was markedly changed by neither hepatic nor renal insufficiency. In contrast, hepatic insufficiency markedly increased plasma levels of t-PA. In rabbits and dogs, plasma levels of lanoteplase were higher than those of alteplase as well as obsd. in rats. The elimination half-lives of lanoteplase were 14-23 min and 8-21 min for alpha-phase and 83-109 min and 66-113 min for beta-phase, resp., in rabbits and dogs. Lanoteplase had a longer half-life than t-PA (2.6-6.5 min and 2.7 min for alpha-phase and 35-53 min and 56 min for beta-phase, resp.) in rabbits and dogs. The CLtot values of lanoteplase were 1/4-1/15 times less than those of t-PA in both animals. In conclusion, as the short half-life of t-PA is mainly explained by its rapid clearance in the liver via receptor mediated process, the clearance of lanoteplase is less mediated via the liver than that of t-PA, resulting longer half-life and prolonged plasma levels compared to t-PA.

Hrelia P, Fimognari C, Maffei F, Vigagni F, Mesirca R, Pozzetti L, Paolini M, Cantelli Forti G. **The genetic and non-genetic toxicity of the fungicide Vinclozolin**. Mutagenesis 1996;11(5):445-53.

The mutagenic/cocarcinogenic potential of the fungicide Vinclozolin was assessed by a comprehensive examination of toxicity mechanisms at both the genetic and the metabolic level. Vinclozolin did not induce any significant increase in chromosomal aberrations in human pheripheral blood lymphocytes cultured in vitro, both in the presence and in the absence of metabolic activation. However, significant dose-related increases in micronucleated erythrocytes (up to 4-fold over the control) were found in the bone marrow cells of mice 24 h after treatment with the fungicide over a range of concentrations from 312.5 to 1250 mg/kg. The morphology and the size of micronuclei induced was suggestive of a predominantly clastogenic mode of action. Several cytochrome P450 (CYP)-dependent reactions have been monitored in liver, kidney and lung microsomes of male and female Swiss Albino CD1 mice in order to ascertain certain toxic non-genetic properties (related to carcinogenesis) of Vinclozolin. It was found to be a selective inducer towards CYP 3A (liver, kidney) and 2E1 (liver), as exemplified by the significant increases of the demethylation of aminopyrine (APND, up to 2.3-fold, female liver), and hydroxylation of p-nitrophenol (pNPH, up to 5.6-fold, male liver). In general, however, Vinclozolin has a complex pattern of induction and suppression of CYP-dependent enzymes, as shown from the reduced expression of various monooxygenases depending upon dose, sex or organ considered. For example, pNPH activity was suppressed in kidney (up to 48% loss, averaged between male and female), whereas ethoxycoumarin O-deethylase was reduced in lung up to 53% in male (at the highest dose). These data were sustained by means of Western immunoblotting using rabbit polyclonal antibodies anti-CYP 3A and 2E1. Northern blotting analysis using CYP 3A1/2 and 2E1 cDNA biotinylated probes showed that the expression of such isozymes is regulated at the mRNA level. Taken together, the findings indicate the clastogenic activity and the possible cotoxic, cocarcinogenic and promoting potential of Vinclozolin.

Humbert H, Cabiac MD, Barradas J, Gerbeau C. **Evaluation of pharmacokinetic studies: is it useful to take into account concentrations below the limit of quantification?** Pharm Res 1996 Jun;13:839-45.
IPA COPYRIGHT: ASHP The usefulness of taking into account samples with values below the limit of quantification for evaluating pharmacokinetics was studied after a single oral dose of drug A in 20 healthy subjects, an oral dose of drug B in 16 healthy subjects, a dose of 2.5, 5, and 10 mg drug C in 18 healthy subjects in a crossover design, and a dose of standard and slow release formulations of drug D in 18 healthy subjects in a crossover design. Under certain conditions, it was possible to get valuable and more reliable kinetic information using concentrations obtained with a poor precision (coefficient of variation >20%). This was especially true for parameters associated with the terminal phase, but also for parameters depending to a lesser extent on the terminal phase. The mean concentration time curve was by far best defined using all of the concentrations.

Itoh K, Nakamura K, Kimura T, Itoh S, Kamataki T. **Molecular cloning of mouse liver flavin containing monooxygenase (FMO1) cDNA and characterization of the expression product: metabolism of the neurotoxin, 1,2,3,4-tetrahydroisoquinoline (TIQ)**. J Toxicol Sci 1997;22(1):45-56.
A mouse liver cDNA clone, MFMO1, coding for a flavin-containing monooxygenase (FMO) was isolated. This cDNA clone encoded a protein of 532 amino acids. Based upon its predicted amino acid sequence, this clone was assumed to belong to the FMO1 subfamily. The deduced amino acid sequence showed 94, 84, 83, and 83% identity with FMO1s of rats, pigs, rabbits and humans, respectively, while it showed only 50-59% identity with human FMO3 and FMO4, rabbit FMO2, FMO3, FMO4 and FMO5, and guinea-pig FMO2. RNA blot analysis showed that the mouse FMO1 was also expressed in the lung and kidney and to lesser extents in the heart, spleen, testis and brain. Mouse FMO1 expressed in yeast showed activities of thiobenzamide S-oxidation, and NADPH oxidation associated with the S- or N-oxidation of chlorpromazine, N,N-dimethylaniline, N,N-dimethyl-hydrazine, imipramine, nicotine, thioacetamide, thiourea and trimethylamine. Moreover, 1,2,3,4-tetrahydroisoquinoline (TIQ), a substance known to induce a parkinsonism-like syndrome in monkeys, was also metabolized by the mouse FMO1. The K(m) values for chlorpromazine, imipramine and TIQ were determined to be 2,4, 16.0, 435 mM, respectively. This is the first report to show that an expressed FMO can metabolize a neurotoxin, TIQ.

Jang JY, Droz PO. **Simulation of toluene in venous blood with a physiologically based pharmacokinetic model: its application to biological exposure index development**. Appl Occup Environ Hyg 1996;11(8):1092-5.
In an effort to redefine biological exposure indices in response to the recent reduction in the American Conference of Governmental Industrial Hygienists threshold limit value for toluene (108883), the use of a physiologically based

pharmacokinetic (PBPK) model for the prediction of venous blood toluene concentrations and its application to the development of biological exposure indices was presented. The PBPK model described consisted of seven compartments representing different tissues. Simulations were performed for a standard man exposed in standard working conditions. Good agreement was seen between the simulation results and actual data obtained in workers. Simulation of blood toluene during an occupational exposure of 50 parts per million, 0.5 and 15 hours after exposure, during a 5 day work week, demonstrated that concentrations were lowest on the first exposure day and increased gradually. The level seen 15 hours after exposure on the first exposure day was only about 50% of the values seen on the other days. With the exclusion of this measurement, venous blood toluene concentrations in morning samples ranged from 0.07 to 0.09 milligram/liter. It was suggested that this range be used as a basis for a biological exposure limit for toluene in blood. The toluene blood level measured 15 hours after exposure was found to reflect the average exposure under different exposure scenarios and various levels of physical activity. Air contamination at the sampling site was found to have serious effects on venous blood concentrations. The authors conclude that PBPK models can provide important information for the establishment of biological exposure indices and that toluene exposure can be monitored by the measurement of toluene in venous blood sampled in the morning before a work shift.

Jonsson EN, Wade JR, Karlsson MO. **Comparison of some practical sampling strategies for population pharmacokinetic studies**. J Pharmacokinet Biopharm 1996 Apr;24:245-63.
IPA COPYRIGHT: ASHP Pharmacokinetic study designs with 1 or 2 samples per visit were compared with respect to precision and bias of the population parameter estimates, the ability to identify the underlying pharmacokinetic model, and the estimation of individual parameter values using simulated or real data sets. Parameter estimates were more biased and imprecise when only 1 sample was taken compared to when 2 samples were obtained. This was true irrespective of the time span between the 2 samples. The ability to identify a more complex model was increased if 2 samples were taken. Two sample designs were generally better with respect to the prediction of individual parameter values. Even minor changes to commonly employed study designs improved the quality and quantity of the information obtained.

Karlsson MO, Beal SL, Sheiner LB. **Three new residual error models for population PK/PD analyses**. J Pharmacokinet Biopharm 1995 Dec;23:651-72.
IPA COPYRIGHT: ASHP Three residual error models for population pharmacokinetic/pharmacodynamic analyses were evaluated using simulated data and a real data set. The models identified the different properties of the residual error for which they were intended: (1) different residual error magnitudes of interreplicate and intersample error, (2) serial correlation of errors, and (3) time-dependent magnitude of error. The examples presented provided qualitative information on how different parameter estimates are influenced when different models for the residual error are used.

Karstadt M, Haseman JK. **Effect of discounting certain tumor types/sites on evaluations of carcinogenicity in laboratory animals**. Am J Ind Med 1997;31(5):485-94.
BIOSIS COPYRIGHT: BIOL ABS. It has been suggested that, for mechanistic reasons, certain tumors found in experimental animals should be discounted when evaluating carcinogenic effects. The questioned tumors are: mouse liver rat thyroid follicular cell, bladder and kidney (male rat), forestomach (mouse and rat, gavage route), and lung (mouse and rat, inhalation of particles). We sought to determine the effects of discounting those tumors on classification of chemicals as carcinogens in animals. We looked at carcinogenicity data for chemicals studied in NCI-ATP bioassays and/or reviewed in IARC monographs and we found that deleting the questioned tumors would have significant impact on evaluations of carcinogenicity in animals. Fifty-six of 234 (24%) chemicals determined to be carcinogenic in the NCI-NTP bioassay program would no longer be considered carcinogenic; 102 (44%) would have weaker evidence of carcinogenic effects. Thirty-three of 361 (9%) chemicals determined by IARC to have limited or sufficient evidence of carcinogenicity would no longer be considered carcinogenic; 119 (33%) would have weaker evidence of carcinogenic effects. Because such a large number of chemicals currently considered carcinogenic would be affected by categorical deletion of tumors and because we are not aware of data that would justify such categorical deletions, it would be preferable to consider mechanistic justifications for discounting tumors

on a case-by-case basis for each individual chemical. Deletion of tumors on a categorical basis has serious implications for regulation of toxic chemicals and for public health.

Koike Y, Tomono Y, Tanaka H, Mineshita S. [**New approach to the drug evaluation: population pharmacokinetic and pharmacodynamic (PK/PD) analysis**]. Rinsho Yakuri 1996;27(4):741-57. (Jpn)
CBAC COPYRIGHT: CHEM ABS A review with 17 refs. The present paper reports a new approach to building a population pharmacokinetic and pharmacodynamic (PK/PD) model and interpreting clin. data. We discuss about a historical background of PK/PD modeling, then, two special parametric models. One is the model to assess the time course of the disease progression and the effects of placebo and actual drug treatment. This model is able to distinguish active drug treatment quant. from placebo effect. The other model is a subject-specific PK/PD model for analgesia using population approach. It is capable of combining characteristic features of clin. trial, i.e., a subject-specific random effect model for a multivariate response that consists of non-continuous component responses, analyze of non-randomly censored longitudinal data and statistics to est. marginal distribution such as Monte Carlo integration. This approach is hopeful about the future in the field of drug development and drug evaluation.

Kress S, Greenlee WF. **Cell-specific regulation of human CYP1A1 and CYP1B1 genes**. Cancer Res 1997;57 (7):1264-9.
BIOSIS COPYRIGHT: BIOL ABS. In this report, we present a characterization of the cell-specific expression of two human cytochrome P450 genes, CYP1A1 and CYP1B1, by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The TCDD-dependent induction of CYP1A1 has been studied extensively and serves as the prototype response for a TCDD-signaling pathway initiated by the reversible binding of TCDD to an intracellular receptor (designated the aryl hydrocarbon (Ah) receptor). CYP1A1 is induced by TCDD to high levels (45-fold increase) in the human hepatoblastoma line HepG2 as compared with the human renal adenocarcinoma line ACHN. In contrast, CYP1B1 is induced selectively in ACHN cells. Cell-specific induction of CYP1A1 and CYP1B1 mRNA correlates with comparable changes in the corresponding proteins and results, at least in part, from transcriptional activation. Characterization of the mechanism(s) for the differential regulation of CYP1A1 was carried out. Nuclear extracts obtained from either cell line following treatment with TCDD displayed equivalent binding to oligonucleotide probes for two dioxin-responsive elements located 5'-ward of the CYP1A1 promoter. This result obtained with broken cell fractions was confirmed by an intact cell DNA protection assay. Possible involvement of negative regulators is suggested by the presence of a negative regulatory element in the 5' flanking region of the CYP1A1 gene and the observed superinduction of CYP1A1 mRNA by cycloheximide in TCDD-treated HepG2 cells. Electromobility shift analysis using negative regulatory element probes, however, did not detect quantitative differences in the binding of nuclear extract proteins obtained from either HepG2 or ACHN cells treated with TCDD. These findings indicate that the ligand-dependent activation and dioxin-responsive element binding of the Ah receptor required for CYP1A1 induction In HepG2 cells also can occur in ACHN cells. We conclude that the repression of TCDD-dependent CYP1A1 induction in ACHN Cells occurs at the level of transactivation in the Ah receptor signal transduction pathway.

Krishnan K, Pelekis M. **Hematotoxic interactions: occurrence, mechanisms and predictability**. Toxicology 1995;105(2-3):355-64.
BIOSIS COPYRIGHT: BIOL ABS. The available data on the binary chemical interactions involving hematotoxicants, particularly organic chemicals causing a reduction in either the number of white/red blood cells or the capacity of hemoglobin to transport oxygen, are limited. These observations are limited to investigations in rodents of the enhancement or attenuation of the hematotoxicity of benzene, dichloromethane and dimethylanilines following prior administration of inducers of CYP 2E1 or co-administration of substrates for this isoenzyme, The relevance of these data on interactions for humans exposed at low concentrations can be assessed only when the mechanism of interaction is understood at a quantitative level, and incorporated within a physiological modeling framework. The present study exemplifies the predictability of the magnitude of binary chemical interactions in humans exposed to low concentrations, by developing a physiological model of the modulation by toluene of dichloromethane-induced carboxyhemoglobinemia. Consistent with the basic biochemical principles, this modeling exercise suggests that, with competitive metabolic inhibition mechanism, the threshold for binary chemical interactions will follow a

downward trend with increasing number of substrates or structurally-similar substances in a mixture. The use of this kind of mechanistic models, along with data from descriptive chemical interaction studies, will form the very basis of mechanistic risk assessment methods for complex chemical mixtures.

Kwon Y. **Effects of diffusional barriers on the extent of presystemic and systemic intestinal elimination of drugs**. Arch Pharm Res 1997;20(1):24-8.
CBAC COPYRIGHT: CHEM ABS A pharmacokinetic model was developed to address the effects of the diffusional barrier between splanchnic bed and enterocytes on the extent of presystemic and systemic intestinal elimination of drugs. The model is composed of 5 compartments, i.e., gut lumen, enterocyte, splanchnic bed, liver, and central compartments. The equations for various pharmacokinetic parameters important for estg. the quant. differences between presystemic and systemic intestinal and hepatic elimination of drugs were derived. A simulation study demonstrated that the diffusional barrier between splanchnic blood and enterocytes can have significant effects on oral bioavailability and systemic clearance of drugs. The model can be useful for a better understanding of the effects of diffusional barrier on the extent of administration-route-dependent intestinal and hepatic elimination of drugs, esp. those with high hydrophilicity and/or charge(s) under physiol. conditions.

Lee Y, Chuong C. **Activation of protein kinase A is a pivotal step involved in both BMP-2- and cyclic AMP-induced chondrogenesis**. J Cell Physiol 1997;170(2):153-65.
CBAC COPYRIGHT: CHEM ABS We studied the roles of protein kinase A (PKA) activation and cAMP response element binding protein (CREB) phosphorylation in chondrogenesis using serum-free chicken limb bud micromass cultures as a model system. We showed the following points: (1) in micromass cultures, activation of PKA enhances chondrogenesis and increases the phosphorylation of CREB; (2) BMP-2, a chondrogenic stimulator, increases PKA activity and the level of phosphorylated CREB (P-CREB); (3) H 8.

Lemon BD, Fondell JD, Freedman LP. **Retinoid X receptor:vitamin D3 receptor heterodimers promote stable preinitiation complex formation and direct 1,25-dihydroxyvitamin D3-dependent cell-free transcription**. Mol Cell Biol 1997;17(4):1923-37.
CBAC COPYRIGHT: CHEM ABS The numerous members of the steroid/nuclear hormone receptor superfamily act as direct transducers of circulating signals, such as steroids, thyroid hormone, and vitamin or lipid metabolites, and modulate the transcription of specific target genes, primarily as dimeric complexes. The receptors for 9-cis retinoic acid and 1,25-dihydroxyvitamin D3 [1,25(OH)2D3], RXR and VDR, resp., as members of this superfamily, form a heterodimeric complex and bind cooperatively to vitamin D responsive elements (VDREs) to activate or repress the transcription of a multitude of genes which regulate a variety of physiol. functions. To directly investigate RXR- and VDR-mediated transactivation, the authors developed a cell-free transcription system for 1,25(OH)2D3 signaling by utilizing crude nuclear exts. and a G-free cassette-based assay. Transcriptional enhancement in vitro was dependent on purified, exogenous RXR and VDR and was responsive to physiol. concns. of 1,25(OH)2D3. The authors found that RXR and VDR transactivated selectively from VDRE-linked templates exclusively as a heterodimeric complex, since neither receptor alone enhanced transcription in vitro. By the addn. of low concns. of the anionic detergent Sarkosyl to limit cell-free transcription to a single round and the use of agarose gel mobility shift expts. to assay factor complex assembly, the authors obsd. that 1,25(OH)2D3 enhanced RXR:VDR-mediated stabilization or assembly of preinitiation complexes to effect transcriptional enhancement from VDRE-linked promoter-contg. DNA

Lieberman R, Mcmichael J. **Role of pharmacokinetic-pharmacodynamic principles in rational and cost-effective drug development**. Ther Drug Monit 1996;18(4):423-8.
IPA COPYRIGHT: ASHP Applications of pharmacologic principles (pharmacokinetic-pharmacodynamic) and modeling methods for drugs in which the evaluation process is guided by and/or identifies significant pharmacokinetic and/or pharmacodynamic variability in drug response are described; case studies from approved new drug applications reviewed at the U.S. Food and Drug Administration (FDA) and employed to illustrate practical uses and places (clinical studies) of exceptional leverage for the efficient application of pharmacokinetic-

pharmacodynamic principles and computer modeling to facilitate demonstration of clinically significant and cost-effective outcomes are presented.

Loizou GD, Tran CL, Anders MW. **Physiologically based pharmacokinetic analysis of the concentration-dependent metabolism of halothane**. Xenobiotica 1997;27(1):87-100.
CBAC COPYRIGHT: CHEM ABS Previous studies with the halothane analog and chlorofluorocarbon replacement 2,2-dichloro-1,1,1-trifluoroethane (HCFC-123) have shown that there are concn.-dependent, sex-specific differences in the rate of uptake during inhalation exposure in rat. Since it is well established that there are sex-specific differences in the control of enzyme activity in drug metab., male and female rats were exposed by inhalation to halothane concns. ranging from 500 to 4000 ppm. A physiol. based pharmacokinetic model describing the concn.-dependent redn. in uptake and metab. of halothane in male and female rats was developed. The in vivo metabolic rate consts. obtained were: for male rats, Km = 0.4 mg litre-1 (2.03 mumol litre-1) and Vmaxc = 9.2 mg kg1 h-1 (46.6 mumol kg1 h-1); for female rats, Km = 0.4 mg litre-1 (2.03 mumol litre-1) and Vmaxc = 10.2 mg kg-1 h-1 (51.7 mumol kg-1 h-1). An equation describing the concn.-dependent decrease of hepatic metab. of halothane successfully simulated the gas-uptake data. Simulation of cumulative urinary excretion of the major metabolite, trifluoroacetic acid, required introduction of a proportionality const. to limit the extent of redn. of halothane metab. to 20% of the amt. of enzyme activity. Good simulation of urinary excretion data was achieved, which was interpreted to indicate that, when only 20% of the enzyme is inactivated, the rate of enzyme resynthesis was adequate to replenish enzyme activity within 24 h. A rapidly reversible, non-biol. inactivation mechanism called 'phys. toxicity' is discussed as a possible explanation of concn.-dependent gas uptake.

Looby M, Weiss M. **Accuracy of noncompartmental pharmacokinetic parameters estimated from bolus injection and steady-state infusion data**. J Pharmacokinet Biopharm 1995 Dec;23635-49.

Ma X, Stoffregen DA, Wheelock GD, Rininger JA, Babish JG. **Discordant hepatic expression of the cell division control enzyme p34cdc2 kinase, proliferating cell nuclear antigen, p53 tumor suppressor protein, and p21Waf1 cyclin-dependent kinase inhibitory protein after WY14,643 ((4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio)acetic acid) dosing to rats**. Molec Pharmacol 1997;51(1):69-78.
BIOSIS COPYRIGHT: BIOL ABS. The hepatocarcinogen and peroxisome proliferator WY14,643 ((4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio)acetic acid) was examined for its ability to induce changes in the intracellular protein expression of hepatic p34cdc2 kinase (CDK1) proliferating cell nuclear antigen (PCNA), p53 tumor suppressor protein, and p21Waf1 CDK inhibiting protein. Young, adult male rats were administered 45 mg-kg/day WY14,643 intraperitoneally for 1, 2, 3, 4, or 5 days or fed diets containing 0% or 0.08% WY14,643 for 1, 2, 3, or 4 weeks. WY14,643 dosing increased concentrations of hepatic proteins of 34- and 37-kDa molecular mass, which were identified through immunoprecipitation as CDK1 and PCNA, respectively. Gel filtration of the hepatic S9 fractions determined by enzyme-linked immunosorbent assay confirmed the increased expression of CDK1 and PCNA immunoreactivity in livers from WY14,643-treated rats. Also, gel filtration revealed that the native CDK1 and PCNA in hepatic S9 from WY14,643-treated rats chromatographed as a major peak with an apparent molecular mass of 70 and 76 kDa, respectively. Immunoblotting of the 70-kDa fraction with anti-CDK1 revealed a single band of molecular mass of 34 kDa. Thus, the CDK1 in the major immunoreactive peak of WY14,643-treated rat liver S9 seems to exist as a heterodimer or homodimer. Immunohistochemistry of formalin-fixed liver demonstrated a cytosolic localization of immunoreactive CDK1 and nuclear localization of immunoreactive PCNA in proliferating cells of WY14,643-treated rat livers. WY14,643 increased hepatic CDK1 content by 1.9-6.3-fold through postdosing days 1-5. Hepatic PCNA content was increased 1.9 -5-fold over the same period. In the 4-week feeding study, CDK1 and PCNA expression were increased at all weekly time points by an average of 15-50-fold, respectively. Furthermore, the dietary administration of 0.08% WY14,643 resulted in sustained, overexpression of hepatic p53 tumor suppressor protein from week 1 through week 4 and of p21Waf1 CDK inhibitory protein from week 3 to week 4.

Martinez E, Moore DD, Keller E, Pearce D, Robinson V, Macdonald PN, Simons SS Jr, Sanchez E, Danielsen M.

The nuclear receptor resource project. Nucleic Acids Res 1997;25(1):163-5.
CBAC COPYRIGHT: CHEM ABS The original Glucocorticoid Receptor Resource (GRR) database has been expanded to include several individual resources as part of a larger project called the Nuclear Receptor Resource (NRR). In addn. to the GRR, the NRR currently features the Thyroid Hormone Receptor Resource, the Androgen Receptor Resource, the Mineralocorticoid Receptor Resource, the Vitamin D Receptor Resource, and the Steroid Receptor Assocd. Proteins Resource. The goal of the NRR project is to provide a comprehensive resource for information on the nuclear receptor superfamily, and to provide a forum for the dissemination and discussion of both published and unpublished material on these proteins. Although the individual resources are managed from different servers, all the files are integrated and can be accessed through the project's Home Page, housed at http://nrr.georgetown.edu/nrr.html. In the near future, it is hoped to expand the project to contain information on other nuclear receptors and to better the electronic publication system. To accomplish this, the involvement of nuclear receptor investigators in the NRR is encouraged.

McClain RM. **Mechanistic considerations for the relevance of animal data on thyroid neoplasia to human risk assessment**. Mutat Res 1995;333(1-2):131-42.
BIOSIS COPYRIGHT: BIOL ABS. There are two basic mechanisms whereby chemicals produce thyroid gland neoplasia in rodents. The first involves chemicals that exert a direct carcinogenic effect in the thyroid gland and the other involves chemicals which, through a variety of mechanisms, disrupt thyroid function and produce thyroid gland neoplasia secondary to hormone imbalance. These secondary mechanisms predominantly involve effects on thyroid hormone synthesis or peripheral hormone disposition. There are important species differences in thyroid gland physiology between rodents and humans that may account for a marked species difference in the inherent susceptibility for neoplasia to hormone imbalance. Thyroid gland neoplasia, secondary to chemically induced hormone imbalance, is mediated by thyroid-stimulating hormone (TSH) in response to altered thyroid gland function. The effect of TSH on cell proliferation and other aspects of thyroid gland function is a receptor mediated process and the plasma membrane surface of the follicular cell has receptors for TSH and other growth factors. Small organic molecules are not known to be direct TSH receptor agonists or antagonists; however, various antibodies found in autoimmune disease such as Graves' disease can directly stimulate or inhibit the TSH receptor. Certain chemicals can modulate the TSH response for autoregulation of follicular cell function and thereby increase or decrease the response of the follicular cell to TSH. It is thus important to consider mechanisms for the evaluation of potential cancer risks. There would be little if any risk for non-genotoxic chemicals that act secondary to hormone imbalance at exposure levels that do not disrupt thyroid function. Furthermore, the degree of thyroid dysfunction produced by a chemical would present a significant toxicological problem before such exposure would increase the risk for neoplasia in humans.

Medinsky MA, Kenyon EM, Seaton MJ, Schlosser PM. **Mechanistic considerations in benzene physiological model development**. Environ Health Perspect 1996;104(Suppl 6):1399-404.
BIOSIS COPYRIGHT: BIOL ABS. Benzene, an important industrial solvent, is also present in unleaded gasoline and cigarette smoke. The hematotoxic effects of benzene in humans are well documented and include aplastic anemia, pancytopenia, and acute myelogenous leukemia. However, the risks of leukemia at low exposure concentrations have not been established. A combination of metabolites (hydroquinone and phenol, for example) may be necessary to duplicate the hematotoxic effect of benzene, perhaps due in part to the synergistic effect of phenol on myeloperoxidase-mediated oxidation of hydroquinone to the reactive metabolite benzoquinone. Because benzene and its hydroxylated metabolites (phenol, hydroquinone, and catechol) are substrates for the same cytochrome P450 enzymes, competitive interactions among the metabolites are possible. In vivo data on metabolite formation by mice exposed to various benzene concentrations are consistent with competitive inhibition of phenol oxidation by benzene. In vitro studies of the metabolic oxidation of benzene, phenol, and hydroquinone are consistent with the mechanism of competitive interaction among the metabolites. The dosimetry of benzene and its metabolites in the target tissue, bone marrow, depends on the balance of activation processes such as enzymatic oxidation and deactivation processes such as conjugation and excretion. Phenol, the primary benzene metabolite, can undergo both oxidation and conjugation. Thus the potential exists for competition among various enzymes for phenol. Zonal localization of phase I and phase II enzymes in various regions of the liver acinus also impacts this

competition. Biologically based dosimetry models that incorporate the important determinants of benzene flux, including interactions with other chemicals, will enable prediction of target tissue doses of benzene and metabolites at low exposure concentrations relevant for humans.

Mehendale HM. **Toxicodynamics of low level toxicant interactions of biological significance: inhibition of tissue repair**. Toxicology 1995;105(2-3):251-66.
BIOSIS COPYRIGHT: BIOL ABS. Because of the complexity of studying the toxicological effects of mixtures of chemicals, much of the mechanistic information has become available through work with binary mixtures of toxic chemicals. Mechanisms derived from studies employing chemicals at individually nontoxic doses are more useful than the mechanisms of interactive toxicity at high doses from the perspective of environmental and public health. Several examples of chemical combinations and interactive toxicity at low doses are now available. Chlordecone-potentiated halomethane hepatotoxicity, where suppression of cell division and tissue repair response permits very high amplification of CCl4 injury culminating in animal mortality, is one such model. Phenobarbital-potentiated CCl4 injury does not lead to animal mortality in spite of much higher liver injury in comparison to the chlordecone + CCl4 model. Much higher stimulation of tissue repair allows the animals to survive despite higher liver injury. Similar interactions have been reported between alcohols and halomethane toxicants. These and other studies have revealed that infliction of toxicant-induced injury is accompanied by a parallel but opposing tissue repair stimulation response which allows the animals to overcome that injury up to a threshold dose. Beyond this threshold, tissue repair response is both diminished and delayed allowing unrestrained progression of injury. Large doses of chemicals can be predictably lethal owing to these two latter effects on tissue repair. Dose-response paradigms in which tissue repair response is measured as a parallel but opposing effect to toxic injury might be useful in more precise prediction of the ultimate outcome of toxic injury in risk assessment. Autoprotection experiments with CCl4, thioacetamide, 2-butoxyethanol and related chemicals as well as heteroprotection against acetaminophen-induced lethality with thioacetamide are examples where tissue repair stimulation has been shown to rescue the animals from massive and normally lethal liver injury. The concept of toxicodynamic interaction between inflicted injury and stimulated tissue repair offers mechanistic opportunity to fine-tune other aspects of human health risk assessment procedure. Tissue repair mechanisms may also offer a mechanistic basis to explain species and strain differences as well as to more accurately assess inter-individual differences in human sensitivity to toxic chemicals. Because tissue repair is affected by nutritional status, assessment of risk from exposure to chemicals without attention to nutritional status may be misleading. Finally, the concept of using maximum tolerated doses (MTDs) in long-term toxicity studies such as cancer bioassays may need to be re-examined. MTDs might be predictably expected to maximally stimulate cell division and it is known that increased cell division is likely to lead to increased number of errors in DNA replication thereby predisposing these animals to cancer. It is clear that detailed studies of toxicodynamic interaction between tissue injury and stimulated tissue repair are likely to yield significant dividends in fine-tuning risk assessment.

Nesnow S, Ross JA, Stoner GD, Mass MJ. **Mechanistic linkage between DNA adducts, mutations in oncogenes and tumorigenesis of carcinogenic environmental polycyclic aromatic hydrocarbons in strain A/J mice**. Toxicology 1995;105(2-3):403-13.
BIOSIS COPYRIGHT: BIOL ABS. Five polycyclic aromatic hydrocarbons (PAHs), benzo(a)pyrene (B(a)P), benzo(b) fluoranthene (B(b)F), dibenz(a,h)anthracene (DBA), 5-methylchrysene (5MC), and cyclopenta(cd)pyrene (CPP) were examined for their lung tumorigenic activities in strain A/J mice, their ability to form PAH-DNA adducts in lung tissues, and their ability to mutate the Ki-ras oncogene in PAH-induced tumors. PAHs dissolved in tricaprylin were administered by single intraperitoneal injection to male strain A/J mice (20 mice/dose) at doses up to 200 mg/kg depending on the PAH. Animals were sacrificed 8 months later and the lungs removed, fixed, and surface adenomas enumerated. DBA produced maximal tumor multiplicity at the highest dose, 10 mg/kg, giving 32.2 lung adenomas per mouse. At 100 mg/kg, B(a)P, B(b)F, 5MC, and CPP gave 12.8, 5.3, 93.1, and 32.2 lung adenomas per mouse, respectively. The dose response data for each PAH was fit to y = 0.6 + bx1.6, where y is the observed mean lung adenomas per mouse at dose x (in mg/kg), 0.6 is the observed background of lung adenomas per mouse, and b is the fitted constant representing the potency of each PAH. Statistical analysis indicated that the fit of the data to the equation was extremely high with adjusted R2 values > 0.985 and small fit standard errors. Based on

this equation, the relative potencies of B(b)F, DBA, 5MC, and CPP compared to B(a)P were PAH (relative activity): DBA (118); 5MC (8.8); CPP (2.9); B(a)P (1.0); B(b)F (0.43). DNA adducts were measured by 32P-postlabeling techniques on DNA from lungs of mice treated with these PAHs. Adducts identified by cochromatography with standards were: from B(a)P, 7R,8S,9S-trihydroxy-10R-(N2-2'-deoxyguanosyl)- 7,8,9,10-tetrahydro- -B(a)P, and two adducts resulting from the metabolic activation of 9-hydroxy-B(a)P and trans-7,8- dihydroxy-7,8-dihydro-B(a)P; from B(b)F, 5-hydroxy-B(b)F9,10-diol-11,12-oxide-2'-deoxyguanosine; from DBA, three adducts from the metabolic activation of trans,trans-3,4,10,11-tetrahydroxy-3,4,10,11-tetrahydro-DBA and two anti-DBA-3,4-diol-1,2- oxide-N2-(2'-deoxyguanosine) adducts; from 5MC, 1R,2S,3S-trihydroxy-4-(N2-2'-deoxyguanosyl)-1,2,3,4-tetrahydro-5M- C; from CPP, four CPP-3,4-oxide-2'-deoxyguanosine adducts. Ki-ras codon 12 mutation analysis of PAH-induced tumors was performed using PCR and dideoxy sequencing methods. Mutations from lung tumors from tricaprylin-treated mice were GGT - GAT, GGT - CGT, and GGT - GTT. DBA produced no mutations in Ki-ras codon 12 above.

Nigrovic V, Banoub A, Diefenbach C, Mellinghoff H, Buzello W. **Onset of the neuromuscular block simulated in an anatomical model**. Br J Clin Pharmacol 1997;43(1):55-63.
CBAC COPYRIGHT: CHEM ABS Aims - The aim of this study was to develop a pharmacodynamic model for nondepolarizing muscle relaxants (neuromuscular blocking agents, NMBAs) based on anatomical, physiol., and pharmacol. considerations and analyze whether the time to onset of the submaximal neuromuscular block (NMB) depends on the affinities of the NMBAs for the postsynaptic receptors or on the pharmacokinetic properties of the NMBAs. Methods - A quant. description of the development of neuromuscular block was achieved by formulating a pharmacodynamic model based on anatomical, physiol. and pharmacol. considerations. The principal characteristics of the model are: (1) Diffusion of the NMBAs out of the capillaries into the interstitial space of muscle and from there into the synaptic space of the motor end plates. (2) Receptor concn. in the synaptic space of 300 muM and the total amt. of receptors in 100 g muscle of between 1.43 x (10-11 to 10-10) mol. (3) Interaction of NMBAs with the receptors defined by the assocn. (kassoc=4 x 108 min-1 x M-1) and dissocn. (kdissoc one of 4, 40, or 400 min-1) rate consts. Results - The simulations demonstrated that different affinities of the NMBAs for the postsynaptic receptors (defined by kassoc/kdissoc) do not influence the onset of the submaximal NMB. The time to the peak submaximal NMB is dependent on the pharmacokinetic properties of the drugs: Those NMBAs that leave plasma rapidly produce the block faster but the fraction of the dose that contributes to the block is small. This fraction is larger for those NMBAs that produce NMB later and, hence, these NMBAs require smaller equieffective doses. Conclusions - We conclude that those muscle relaxants that produce neuromuscular block rapidly require larger equieffective doses due to their more rapid initial disappearance from plasma.

O'Flaherty EJ. **Pharmacokinetics, pharmacodynamics, and prediction of developmental abnormalities**. Reprod Toxicol 1997;11(2-3):413-6.
CBAC COPYRIGHT: CHEM ABS A review with 28 refs. The purpose of this article is to review the application of pharmacokinetics and pharmacodynamics to risk assessment for developmental toxicants and to the development of predictive models of developmental toxicity. Physiol. based pharmacokinetic models require knowledge of the anat. and physiol. changes taking place during pregnancy and of how the potential developmental toxicant is distributed into differentiating and developing fetal tissues. Physiol. based pharmacokinetic models of varying levels of complexity have been proposed for rodent and human gestation. Models of pharmacodynamics behavior are less well developed, although structure-activity relationships have led to useful insights for certain specific classes of developmental toxicants. Development of biol. based dose-response models, which integrate pharmacokinetics and pharmacodynamics, in its early phases. Although their development has proven to be complex, biol. based dose-response models have the potential to form the basis for a broader understanding of mechanisms of developmental toxicity and for broad predictive utility in developmental toxicity

. Panse J, Hipp ML, Bauer G. **Fibroblasts transformed by chemical carcinogens are sensitive to intercellular induction of apoptosis: implications for the control of oncogenesis**. Carcinogenesis 1997;18(2):259-64.
The ability of neighbouring normal cells to inhibit proliferation of transformed cells is regarded as the classical mode of intercellular control of potential tumour cells. This mechanism, however, only controls the pool size of

transformed cells, but does not impair their survival. We have recently shown that cells transformed by biological agents are subject to a novel control system: transforming growth factor beta (TGF-beta) induces normal cells to release factors that mediate apoptosis specifically in transformed cells. Here we show that cells transformed by chemical carcinogens are also subject to this dominant control mechanism. The number of foci induced by methylcholanthrene, N-methyl-N'-nitro-N-nitrosoguanidine or quercetin was significantly reduced when the cultures were treated with TGF-beta. Established lines of chemically transformed cells proved to be sensitive to induction of apoptosis by neighbouring normal cells in the presence of TGF-beta. This finding demonstrates that sensitivity to induction of apoptosis is a general feature of transformed cells, irrespective of the transforming agent. It is particularly relevant for chemical carcinogenesis. As transformed cells were shown to trigger induction of their own apoptosis, the acquisition of resistance to this process may be a central regulatory step in carcinogenesis in vitro and possibly also in vivo. This study may help to elucidate mechanisms that protect transformed cells at an early stage of tumour progression that has until now not been the focus of investigation.

Pelekis M, Krishnan K. **Assessing the relevance of rodent data on chemical interactions for health risk assessment purposes: a case study with dichloromethane-toluene mixture**. Regul Toxicol Pharmacol 1997;25 (1):79-86.
Several descriptive studies have reported the occurrence of infra-additive and supra-additive toxic interactions in rodents given high doses of chemicals by routes different from anticipated human exposures. In order to assess the relevance of such rodent data on chemical interactions for humans, the route, species, and dose extrapolations need to be conducted on the basis of proven/hypothetical interaction mechanisms. The present study initially developed a physiologically based model of the toxicological interaction reported in rats receiving high oral doses of dichloromethane (DCM) and toluene (TOL). This predictive model was then used to asses the relevance of DCM-TOL interaction for humans exposed to threshold limit values (TLVs) of these chemicals, following the conduct of the various, essential extrapolations (i.e., rat to human, oral to inhalation, high dose to low dose). The interaction modeling approach involved (I) obtaining validated rat and human physiologically based pharmacokinetic (PBPK) models for TOL and DCM from the literature, and (ii) linking them via the modified Michaelis-Menten equation accounting for hypothetical mechanisms of interactions (no interaction, competitive inhibition, noncompetitive inhibition, and uncompetitive inhibition). Of the various interaction mechanisms investigated, the noncompetitive and uncompetitive metabolic inhibitions were found to adequately describe the reduction of carboxyhemoglobinemia (COHB) observed in rats during combined exposures (18.8 mmol/kg TOL, +6.2 mmol/kg DCM, po; 0.005 mmol/kg TOL, ip +5000 ppm DCM, 1 hr). The simulation model, based on noncompetitive and uncompetitive inhibition mechanisms, suggests that only < 10% reduction in the area under the COHB vs time curve (AUCCOHB) is likely to occur in humans exposed to the current TLVS of DCM and TOL (Compared to AUCCOHB resulting from an 8-hr exposure to TLV of DCM alone). The present modeling approach, based on hypothetical mechanisms of interaction, then indicates that rodent data on DCM-TOL interaction are not relevant for humans, particularly with respect to the COHB effect. The application of this kind of a predictive modeling approach should be useful in screening the available reports on chemical interactions for identifying those of greater concern at relevant human exposure levels (RFD, RFC, TLV).

Piselli P, Vendetti S, Poccia F, Cicconi R, Mattei M, Bolognesi A, Stirpe F, Colizzi V. **In vitro and in vivo efficacy of heat shock protein specific immunotoxins on human tumor cells**. J Biol Regul Homeost Agents 1995;9 (2):55-62.
The presence of heat shock proteins (HSPs) on the surface of tumor cells suggested the possibility of using stress proteins as immunological target for specific immunotoxins (ITs). Flow cytometry analysis showed that U937 cells constitutively express both 28 and 60 kDa HSP in vitro, while the HPC-4 cells only express surface HSPs when grown in vivo, i.e. explanted from SCID mice. Incubation of U937 cells with monoclonal antibodies against 28 or 60 kDa HSP, and then with an immunotoxin consisting of a goat anti-mouse antibody linked to the ribosome inactivating protein Saporin-6 specifically inhibits cell proliferation in vitro. Moreover, an anti-HSP60 immunotoxin prepared by direct linking of the specific monoclonal antibody (MoAb) ML30 to saporin was able to inhibit the proliferation of the U937 line in vitro, and tumor growth in SCID mice bearing the human pancreatic carcinoma line HPC-4 in vivo. Finally, low expression of HSPs on the membrane of peripheral blood mononuclear cells, and their

resistance to the toxic effect exerted by anti-HSP immunotoxins, suggest further evaluation of the possible applications of anti-HSP immunotoxins for HSP+tumors.

Ploemen JP, Wormhoudt LW, Haenen GR, Oudshoorn MJ, Commandeur JN, Vermeulen NP, De Waziers I, Beaune PH, Watabe T, Van Bladeren PJ. **The use of human in vitro metabolic parameters to explore the risk assessment of hazardous compounds: the case of ethylene dibromide**. Toxicol Appl Pharmacol 1997;143 (1):56-69.
Ethylene dibromide (1,2-dibromoethane, EDB) is metabolized by two routes: a conjugative route catalyzed by glutathione S-transferases (GST) and an oxidative route catalyzed by cytochrome P450 (P450). The GST route is associated with carcinogenicity. An approach is presented to use human purified GST and P450 enzymes to explore the importance of these metabolic pathways for man in vivo. This strategy basically consists of four steps: (i) identification of the most important isoenzymes in vitro, (ii) scaling to rate per milligram cytosolic and microsomal protein, (iii) scaling to rate per gram liver, and (iv) incorporation of data in a physiologically based pharmacokinetic (PBPK) model. In the first step, several GST isoenzymes were shown to be active toward EDB and displayed pseudo-first-order kinetics, while the EDB oxidation was catalyzed by CYP2E1, 2A6, and 2B6, which all displayed saturable kinetics. In the second step, the predictions were in agreement with the measured activity in a batch of 21 human liver samples. In the third step, rat liver P450 and GST metabolism of EDB was predicted to be in the same range as human metabolism (expressed per gram). Interindividual differences in GST activity were modeled to determine extreme cases. For the most active person, an approximately 1.5-fold increase of the amount of conjugative metabolites was predicted. Lastly, it was shown that the GST route, even at low concentrations, will always contribute significantly to total metabolism. In the fourth step, a PBPK model describing liver metabolism after inhalatory exposure to EDB was used. The saturation of the P450 route was predicted to occur faster in the rat than in man. The rat was predicted to have a higher turnover of EDB from both routes. Nevertheless, when all data are combined, it is crucial to recognize that the GST remains significantly active even at low EDB concentrations. The limitations and advantages of the presented strategy are discussed.

Plowchalk DR, Andersen ME, Bogdanffy MS. **Physiologically based modeling of vinyl acetate uptake, metabolism, and intracellular pH changes in the rat nasal cavity**. Toxicol Appl Pharmacol 1997;142(2):386-400.
Chronic inhalation exposure to vinyl acetate (VA) causes lesions in the nasal cavity of the rat. This effect appears to be related to tissue exposure to either acetaldehyde (AAld) or acetic acid (AA) metabolites of VA or both. A physiologically based pharmacokinetic model was constructed to describe the deposition of VA in the nasal cavity of the rat and provide estimates of regional tissue exposure to VA, AAld, and AA. Since formation of AA in the nasal tissue should cause intracellular acidification, a submodel which describes free intracellular hydrogen ion concentration and intracellular pH (pHi) changes was linked to the VA model. The dosimetry model was applied to data from a series of experiments designed to measure the uptake and metabolism of VA in the isolated upper respiratory tract of the rat at exposure concentrations ranging from 73 to 2190 ppm. Extraction of VA from the nasal cavity was nonlinear with respect to exposure concentration and ranged from 36 to 94%, with the greatest deposition occurring at the lowest VA concentrations. Pretreatment with bis(p-nitrophenyl)phosphate, an inhibitor of carboxylesterases, significantly reduced fractional deposition of VA compared to naive rats exposed to similar VA concentrations. The best model fits for VA extraction and AAld appearance were achieved when a second carboxylesterase isozyme, with high-affinity characteristics, was included. Simulations of 6-h inhalation exposures to VA predicted that the order of nasal tissue exposures will be to AA > AAld > VA. In addition, based on measured tissue hydrolysis rates, sufficient acid should be formed by the metabolism of VA to cause significant changes in pHi. VA exposures of 200 and 600 ppm were predicted to result in a pHi of less than 7.2 and 6.7, respectively. This model provides nasal dosimetry estimates needed to develop mechanistically based risk assessment approaches for human exposures to VA vapor.

Polasa K, Krishnaswamy K. **Reduction of urinary mutagen excretion in rats fed garlic**. Cancer Lett 1997;114(1-2):185-6.
Naturally occurring substances of plant origin are known to possess antimutagenic potential. Garlic (Allium sativum) was fed to rats in dried powdered form at 0.1%, 0.5% and 1% concentrations in their diet for 4 weeks. At the end of

the experiment benzo[a]pyrene (1 mg/rat) was injected intraperitoneally and 24-h urine was collected from the rats. Urinary mutagens were quantitated by the Salmonella typhimurium assay. There was a significant reduction in the excretion of urinary mutagens by carcinogen-exposed rats fed garlic. Further, there was a stimulation in the activities of liver cytosolic glutathione-S-transferase and liver and lung quinone reductases. The study suggested that the antimutagenic potential of garlic may be mediated through induction of detoxification enzymes in target tissues

. Porter DW, Nelson VC, Fivash MJ Jr, Kasprzak KS. **Mechanistic studies of the inhibition of MutT dGTPase by the carcinogenic metal Ni(II)**. Chem Res Toxicol 1996;9(8):1375-81.
Promutagenic 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG) levels are increased in DNA of animals exposed to carcinogenic metals, such as Ni(II). Besides being generated directly in genomic DNA, 8-oxo-dG may be incorporated there from 8-oxo-7,8-dihydro-2'-deoxyguanosine 5'-triphosphate (8-oxo-dGTP), a product of oxidative damage to the nucleotide pool. The Escherichia coli dGTPase MutT, and analogous dGTPases in rats and humans, have been suggested as a defense against such incorporation because they hydrolyze 8-oxo-dGTP to 8-oxo-7,8-dihydro-2'-deoxyguanosine 5'-monophosphate (8-oxo-dGMP). MutT and its mammalian counterparts are Mg(II)-dependent enzymes. Ni(II), in turn, is known to interact antagonistically with Mg(II) in biological systems. Thus, we hypothesized that Ni(II) might inhibit the activity of MutT. As an initial examination of this hypothesis, we conducted enzyme kinetic studies of MutT to determine the effect of Ni(II) on MutT activity and the mechanisms involved. As found, Ni(II) inhibited MutT in a concentration-dependent manner when either dGTP or 8-oxo-dGTP was the nucleotide substrate. Ni(II) was determined to be an uncompetitive inhibitor of MutT with respect to Mg(II) when dGTP was the substrate, with apparent Ki of 1.2 mM Ni(II), and a noncompetitive inhibitor with respect to Mg(II) when 8-oxo-dGTP was the substrate, with apparent Ki of 0.9 mM Ni(II). Hence, the two metal cations did not compete with each other for binding at the MutT active site. This makes it difficult to predict Ni(II) effects on 8-oxo-dGTPases of other species. However, based upon the amino acid sequences of human and rat MutT-like dGTPases, their capacity for Ni(II) binding should be greater than that of MutT. Whether this could lead to stronger inhibition of those enzymes by Ni(II), or not, remains to be investigated.

Proost JH, Wierda JM, Meijer DK. **Extended pharmacokinetic/pharmacodynamic model describing quantitatively the influence of plasma protein binding, tissue binding, and receptor binding on the potency and time course of action of drugs**. J Pharmacokinet Biopharm 1996 Feb;24:45-77.
IPA COPYRIGHT: ASHP An extended pharmacokinetic/pharmacodynamic model that considers the effect of binding of the drug to plasma proteins and to tissue binding sites in a peripheral compartment and receptor binding in the effect compartment is presented; the model is characterized by the following parameters: the exit rate constant of unbound drug from the effect compartment, the ratio of the unbound clearances to and from the effect compartment, the fraction of drug in effect compartment that is not bound to nonspecific binding sites, the equilibrium dissociation constant of drug-receptor binding, and the concentration of receptor binding sites in effect compartment.

Purves RD. **Multiple solutions, illegal parameter values, local minima of the sum of squares, and anomalous parameter estimates in least-squares fitting of the two compartment pharmacokinetic model with absorption**. J Pharmacokinet Biopharm 1996 Feb;24:79-101.
IPA COPYRIGHT: ASHP The sources of confusion and difficulty in fitting the two compartment pharmacokinetic model with absorption, including false minima and anomalous parameter estimates, the effect of parameterization on the least-squares method, and the generation of anomalous data sets, are presented.

Rodriguez Larrea J. [**Study of the population variability in pharmacokinetics and pharmacodynamics. Part 1. General concepts**]. Cienc Pharm 1996;6(2):96-106. (Spa)
IPA COPYRIGHT: ASHP The analysis of pharmacokinetic or pharmacodynamic data from a population point of view, beginning with the design of the study, collection of data, elaboration of the pharmacostatistical model, application of a population method, and finishing with the validation of the results, is described.

Roe DJ, Karol MD. **Averaging pharmacokinetic parameter estimates from experimental studies: statistical theory and application**. J Pharm Sci 1997;86(5):621-4.
CBAC COPYRIGHT: CHEM ABS In most exptl. pharmacokinetic studies, parameter ests. are computed sep. for each subject, then averaged across subjects. Av. estimators for ratios and functions of parameters are often of interest; examples include half-life and clearance. For these parameters, recommendations regarding averaging using the arithmetic vs. the harmonic mean have been based on computer simulations.1-3. The goal in this paper was to demonstrate that these empirically generated results can be derived using approxns. for the expected values of reciprocals and ratios. The authors first consider estg. the reciprocal of a parameter, and predict the earlier simulation results for half-life. The authors addnl. predict results for clearance when computed as dose divided by area under the curve. Next, the authors consider estg. the ratio of 2 parameters, and predict the earlier simulation results for clearance in a first-order exponential model. As a further example, the authors predict results for the mean residence time in noncompartmental anal. These approxns. provide a unifying approach that can be used to det. optimal summary estimators, without the need for extensive computer simulations.

Rosenbaum SE, Lam J. **Bioequivalence parameters of parent drug and its first-pass metabolite: comparative sensitivity to sources of pharmacokinetic variability**. Drug Dev Ind Pharm 1997;23(3):337-44.
CBAC COPYRIGHT: CHEM ABS The relative susceptibilities of a drug and its first-pass metabolite to various forms of pharmacokinetic variability were studied. Plasma concns. of both species were simulated under conditions of interindividual variability in intrinsic hepatic clearance, intraindividual variability in hepatic clearance, and/or a concn.-dependent model for assay error. In comparison to the metabolite, the plasma concns. of the parent drug displayed a heightened sensitivity to all forms of error. The bioequivalence parameters, AUC, Cmax, and Tmax of the parent drug and the metabolite were detd. from an abbreviated data set. Compared to the metabolite, the AUC and Cmax of the parent drug were much more sensitive to the added variability. The Tmax of both species displayed similar variability. For a given level of manufacturer risk, a much larger group of subjects would be necessary to demonstrate bioequivalence with respect to the drug than with respect to the metabolite.

Rowland M, Mclachlan A. **Pharmacokinetic considerations of regional administration and drug targeting: influence of site of input in target tissue and flux of binding protein**. J Pharmacokinet Biopharm 1996;24 (4):369-87.
CBAC COPYRIGHT: CHEM ABS Hunt et al. introduced the concept of the Drug Targeting Index (DTI) to quantify the gain assocd. with regional drug administration and targeting and showed that for the ideal case of all drug first reaching the target DTI = 1 + CLs/(QT(1-ET)) where CLs is the total clearance of drug from the body (including the target tissue), QT is the target blood flow and ET is the steady-state extn. ratio of the drug in the target. In the model they portrayed the tissue as a homogeneous organ. A more general pharmacokinetic model has been developed that takes into account the three anatomical spaces (vascular, interstitial, and intracellular) of the target organ or tissue and that, in addn. to unbound drug permeating the vascular and cellular membranes, protein-bound drug can also flux between the vascular and interstitial spaces. Elimination of unbound drug can take place from the cellular and interstitial spaces. An important parameter influencing the DTI is shown to be the fraction of targeted dose that is eliminated there before it reaches the systemic circulation, fT. Equations have been developed showing the relationship between fT and ET and for DTI when drug is administered at the various sites within the tissue and under a variety of conditions. Only when drug is administered into the target arterial blood stream or when distribution of drug within the target tissue is perfusion rate-limited, does fT=ET and DTI = 1 + CLs/(Qr (1 - Er)). Otherwise consideration needs to be given to the permeabilities of both the unbound and bound drug and site of target administration, interstitial or intracellular. Then fT is greater than Er and DTI is greater than that expected had perfusion-rate limited distribution prevailed. The max. benefit in DTI is seen for a drug of low cellular permeability but high cellular intrinsic clearance administered intracellularly.

Roy A, Weisel CP, Gallo M, Georgopoulos P. **Studies of multiroute exposure/dose reconstruction using physiologically based pharmacokinetic models**. J Clean Technol Environ Toxicol Occup Med 1996;5(4):285-95.
CBAC COPYRIGHT: CHEM ABS Volatile org exposure reconstruction pharmacokinetic model;Air pollution Volatile

org. multiroute exposure/dose reconstruction using physiol. based pharmacokinetic models;Volatile organic compounds Volatile org. multiroute exposure/dose reconstruction using physiol. based pharmacokinetic models.

Ryffel B. **Impact of knockout mice in toxicology**. Crit Rev Toxicol 1997;27(2):135-54.
CBAC COPYRIGHT: CHEM ABS A review with 120 refs. Knockout mice obtained by homologous recombination technol. may be valuable tools for in vivo investigations in toxicopathogenesis. A short review is given on the phenotype of mice with distinct deletions of cytokines and related genes. The application of these mice in pharmacol. and toxicol. research is discussed, with emphasis in endotoxic shock, hepatic toxicity, and myelotoxicity. The use of such knockout mice will be valuable for mechanistic studies in toxicol.

Semino G, Lilly P, Andersen ME. **A pharmacokinetic model describing pulsatile uptake of orally-administered carbon tetrachloride**. Toxicology 1997;117(1):25-33.
Many rodent bioassays have been conducted using oral gavage for delivery of test chemicals. Highly lipophilic compounds are generally administered to rodents dissolved in corn oil, a dosing vehicle shown to influence xenobiotic toxicity, carcinogenicity and pharmacokinetics by altering chemical absorption processes. In this paper, we present a multi-compartmental description of the gastrointestinal (GI) tract linked to a physiologically based pharmacokinetic (PB-PK) model to describe the complex oral uptake of carbon tetrachloride (CCl4) administered in corn oil and 0.25% Emulphor. The GI submodel was described using a series of subcompartments, each subcompartment described with an absorption constant (Ka, 1/h), a bioavailability term (A, unitless), and a compartment emptying time (T, h). The model was parameterized by fitting multi-peak blood and exhaled breath chamber concentration-time profiles following oral gavage of CCl4 in corn oil and aqueous vehicles to male Fischer 344 rats. Successful fitting of experimental data was accomplished by varying values of Ka, A, and T until adequate fits were obtained. Values of Ka and A required to fit data from aqueous gavage were greater than corn oil. Utilization of the multi-compartmental GI tract submodel provided increased precision in fitting complex oral uptake profiles compared to previously used one- and two-compartment oral uptake models. This model provides estimates of absorption rate constants and bioavailabilities as well as providing a framework for generation of more complete, physiologically-realistic descriptions of oral absorption.

Simmons JE. **Application of physiologically based pharmacokinetic modelling to combination toxicology**. Food Chem Toxicol 1996;34(11-12):1067-73.
BIOSIS COPYRIGHT: BIOL ABS. Non-additive toxicity has been demonstrated in laboratory animals for a large number of temporally separated or concurrent multiple chemical exposures. These exposures are typically at concentrations higher than those found in the environment, leading to the question of the applicability of the results to the human situation. Physiologically based pharmacokinetic (PBPK) modelling has been applied successfully to single chemicals; its utility for extrapolation across species and dose has been demonstrated. Use of PBPK modelling in the study of chemical mixtures is increasing although still limited. The use of PBPK modelling by various investigators in the field of combination toxicology is reviewed. PBPK modelling has been used to examine: the role of increased metabolism in non-additive toxicity resulting from temporally separated exposures; the influence of the time interval separating two chemical exposures; and the role of inhibition of metabolism in concurrent exposure to two chemicals. In summary, development of a PBPK or PBPK/pharmacodynamic model for a combined exposure provides a basis for extrapolation across species, route and dose, and a useful tool for risk assessment.

Staretz ME, Murphy SE, Patten CJ, Nunes MG, Koehl W, Amin S, Koenig LA, Guengerich FP, Hecht SS. **Comparative metabolism of the tobacco-related carcinogens benzo[a]pyrene, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, and N'- nitrosonornicotine in human hepatic microsomes**. Drug Metab Dispos 1997;25,(2):154-62.
We compared the metabolism in human hepatic microsomes of three tobacco smoke carcinogens believed to be involved in the induction of cancer in humans: benzo[a]pyrene (BaP),4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and N'-nitrosonomicotine (NNN). The metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), a

major metabolite of NNK, was also investigated. Although the metabolism of some of these compounds by human enzymes or tissue preparations has been previously examined in some studies, they have never been compared in the same human hepatic samples. Moreover, there have been no previous reports of NNAL metabolism by human tissues or enzymes. The tritium-labeled carcinogens (3 microM) were incubated with 10 different human hepatic microsomal preparations and cofactors for 10-20 min, and the products were analyzed by radioflow HPLC. NNN was the best substrate for oxidative metabolism, with the 5'-hydroxylation pathway being the predominant one observed (mean +/- SD = 31 +/- 17 pmol/min/mg protein). alpha-Hydroxylation of NNK by the methylene and methyl hydroxylation metabolic activation pathways was the next fastest reaction, with rates of 3.1 +/- 1.9 and 3.3 +/- 1.1 pmol/min/mg protein, respectively. Metabolism of BaP resulted in the formation of dihydrodiols and phenols; trans-7,8-dihydro-7,8-dihydroxy-BaP, its major proximate carcinogen, was formed at a rate of 1.1 +/- 0.61 pmol/min/mg protein. alpha-Hydroxylation of NNAL proceeded at a rate of 0.53 +/- 0.26 pmol/min/mg protein. The results of this study demonstrate that human hepatic microsomes metabolize all of these tobacco carcinogens resulting in a substantial stream of electrophilic intermediates capable of binding to DNA. The relative rates of oxidative metabolism to electrophiles or their precursors were NNN > NNK > BaP > NNAL. Correlation studies indicated involvement of cytochrome P4502A6 in the 5'-hydroxylation of NNN and cytochrome P4503A4 in the alpha-methylene hydroxylation and pyridine-N-oxidation of NNK and NNAL. The results of this study provide the first data on the comparative metabolism of these important carcinogens in human hepatic microsomes.

Stiborova M, Hansikova H, Frei E. **Metabolism of carcinogenic N-nitroso-N-methylaniline by purified cytochromes P450 2B1 and P450 2B2**. Cancer Lett 1996;110(1-2):11-7.
N-Nitroso-N-methylaniline (NMA) is an esophageal carcinogen in the rat. The in vitro enzymatic metabolism of NMA was investigated using cytochromes P450 2B1 and P450 2B2, isolated from liver microsomes of rats pretreated with phenobarbital (PB), reconstituted with NADPH-cytochrome P450 reductase and dilauroylphosphatidylcholine. Formaldehyde is produced by both cytochromes P450 (P450). NMA is a better substrate for P450 2B1 than for P450 2B2. The maximal velocity (Vmax) values are 3.3 and 1.6 nmol HCHO/min per nmol P450 for P450 2B1 and P450 2B2, respectively. Beside formation of formaldehyde, aniline and p-aminophenol (p-AP) are found to be metabolites formed from NMA by both P450 isoenzymes. P450 2B1 also affords phenol, while none was found with the P450 2B2 isoenzyme. Phenol formation presumably arose from direct alpha-C-hydroxylation of NMA via a benzenediazonium ion (BDI) intermediate. The results suggest strongly that P450 2B1 catalyzes both alpha-C-hydroxylation and denitrosation of NMA while P450 2B2 catalyzes only denitrosation.

Swales NJ, Caldwell J. **Phase 1 and 2 metabolism in freshly isolated hepatocytes and subcellular fractions from rat, mouse, chicken and ox livers**. Pestic Sci 1997;49(3):291-9.
BIOSIS COPYRIGHT: BIOL ABS. In toxicological studies hepatocytes offer an excellent alternative to whole-animal experiments, provided their metabolic competence has been established. We have compared Phase 1 and 2 metabolism in rat, mouse, chicken and ox liver microsomes and cytosol with freshly isolated hepatocytes. The relative amounts of total cytochrome P450 in microsomes and hepatocytes were equivalent. Rat liver had the highest P450 content while chicken liver had the lowest content (148.2(:75.7) and 20.6(:11.5) pmol mg-1 hepatocellular protein, respectively). The metabolism of testosterone was assessed to determine selective cytochrome P450 isoenzyme activities. Only two metabolite products were common to all four species, namely 6beta-hydroxytestosterone (6beta-OHT) and androstenedione (ASD), which co-eluted with 6-dehydrotestosterone (6DHT). 16alpha-OHT was present in all incubations except for ox microsomes. The rate of metabolism of testosterone was generally lower in microsomes than hepatocytes, w the exception of the ox, but the pattern and quantity of metabolite formation was similar. The quantity of total products formed was 15- to 27-fold higher in rat and mouse livers than in chicken or ox. The major product formed in freshly isolated hepatocytes from mice and chickens was ASD/6DHT which accounted for 60% and 76% of the total metabolites, respectively. ASD/6DHT formation accounted for only 33% and 17% of the total metabolites formed by rat and ox hepatocytes, respectively. 2alpha-OHT production occurred in rat and mouse hepatocytes (14% of the total metabolites in rat and 7% in mouse hepatocytes) but was lacking in chicken or ox cells. The stability of P450 isoforms in culture was species-dependent. Rat and mouse hepatocyte cultures lost 54% and 31% of their initial P450 content after 72 h, while there was no loss in chicken hepatocytes over the same period. There was a good correlation between the relative

glutathione S-transferase (GST) activities in cytosol and freshly isolated hepatocytes. Mouse liver exhibited highest GST activity (664.2(:203.5)) compared with rat, chicken or ox (320.4(:64.0), 341.5(:13.9) and 256.3(:109.9)nmol min-1 mg-1 cytosolic protein, respectively).

Sweeney LM, Schlosser PM, Medinsky MA, Bond JA. **Physiologically based pharmacokinetic modeling of 1,3-butadiene, 1,2-epoxy-3-butene, and 1,2:3,4-diepoxybutane toxicokinetics in mice and rats**. Carcinogenesis 1997;18(4):611-25.
1,3-Butadiene (BD) is a more potent tumor inducer in mice than in rats. BD also shows striking differences in metabolic activation, with substantially higher blood concentrations of 1,2:3,4-diepoxybutane (butadiene diepoxide; BDE) in BD-exposed mice than in similarly exposed rats. The objective of this study was to develop a single mechanistic model structure capable of describing BD disposition in both species. To achieve this objective, known pathways of 1,2-epoxy-3-butene (butadiene monoepoxide; BMO) and BDE metabolism were incorporated into a physiologically based pharmacokinetic model by scaling rates determined in vitro. With this model structure, epoxide clearance was underestimated for both rats and mice. Improved simulation of blood epoxide concentrations was achieved by addition of first-order metabolism in the slowly perfused tissues, verified by simulation of data on the time course for BMO elimination after i.v. injection of BMO. Blood concentrations of BD were accurately predicted for mice and rats exposed by inhalation to constant concentrations of BD. However, if all BD was assumed to be metabolized to BMO, blood concentrations of BMO were overpredicted. By assuming that only a fraction of BD metabolism produces BMO, blood concentrations of BMO could be predicted over a range of BD exposure concentrations for both species. In vitro and in vivo studies suggest an alternative cytochrome P-450-mediated pathway for BD metabolism that does not yield BMO. Including an alternative pathway for BD metabolism in the model also gave accurate predictions of blood BDE concentrations after inhalation of BD. Blood concentrations of BMO and BDE observed in both mice and rats are best explained by the existence of an alternative pathway for BD metabolism which does not produce BMO.

Thorgeirsson UP, Snyderwine EG, Gomez DE, Adamson RH. **Dietary heterocyclic amines as potential human carcinogens: experimental data from nonhuman primates**. In Vivo 1996;10(2):145-52.
During the cooking of meats, several highly potent mutagenic heterocyclic amines (HCAs) are produced. To date, 10 HCAs have been shown to be carcinogenic in rodents, and one HCA, 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), has been shown to be a potent hepatocarcinogen in nonhuman primates. In this report, we discuss the role of metabolic activation and DNA adduct formation in the carcinogenicity of HCAs, especially in nonhuman primates. The potent hepatocarcinogenicity of IQ in cynomolgus monkeys appears to be associated with the in vivo metabolic activation of IQ and the formation of hepatic DNA adducts. Notably, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), is poorly metabolically activated in monkeys and lacks the potency of IQ to induce hepatocellular carcinoma. Ongoing studies with 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), indicate that PhIP is metabolically activated in monkeys and is a likely carcinogen in this species. We further compared human, rat, and cynomolgus monkey hepatic microsomes for their abilities to metabolically activate various HCAs to mutagens. Our in vitro results show that humans, relative to rats or cynomolgus monkeys, have a good capacity to metabolically activate the HCAs. These findings support the concept that humans are likely to be susceptible to the carcinogenic effects of HCAs.

Toyokuni S, Luo XP, Tanaka T, Uchida K, Hiai H, Lehotay DC. **Induction of a wide range of C2-12 aldehydes and C7-12 acyloins in the kidney of Wistar rats after treatment with a renal carcinogen, ferric nitrilotriacetate**. Free Radical Biol Med 1997;22(6):1019-27.
BIOSIS COPYRIGHT: BIOL ABS. An iron chelate, ferric nitrilotriacetate (Fe-NTA), induces renal proximal tubular necrosis associated with lipid peroxidation and oxidative DNA damage that finally leads to a high incidence of renal cell carcinoma in rodents. In the present study, we investigated what kinds of C2-12 saturated and unsaturated aldehydes and C7-12 acyloins, metabolites of saturated aldehydes, are produced in the kidney and liver within 24 h after single ip administration of 15 mg Fe/kg of Fe-NTA, or after repeated (1 or 3 wk) ip administration of 5 - 10 mg Fe/kg of Fe-NTA. Amounts of twenty one aldehydes and five acyloins were determined by capillary column gas chromatography-negative-ion chemical ionization mass spectrometry with ammonia as reagent gas. Most of the

aldehydes and all the acyloins measured revealed a significant dose-dependent increase 1 to 3 h after single administration in the kidney, among which 4-hydroxy-2-nonenal (HNE) showed the highest increase (27.3-fold) and malondialdehyde (MDA) was the most abundant aldehyde (2.40 nmol/100 mg wet tissue). In the liver, however, the increase in aldehydes and acyloins was less prominent. After repeated administration of Fe-NTA, only 9 aldehydes (ethanal; furfural; trans,trans-2,4-heptadienal; nonanal; trans-2,cis-6-nonadienal; HNE; decanal; trans-4,cis-4-decenal; MDA) and 4 acyloins (3-hydroxyheptan-2-one; 3-hydroxyoctan-2-one; 3-hydroxynonan-2-one; 3-hydroxydodecan-2-one) showed a significant increase. Immunohistochemistry further demonstrated an increased amount of HNE-modified and MDA-modified proteins in the renal proximal tubules after repeated Fe-NTA administration. Some of the aldehydes measured such as HNE and MDA are reportedly cytotoxic, genotoxic and mutagenic. Accumulation of these aldehydes may play a role in this renal carcinogenesis model.

Uno K, Aoki T, Ueno R, Maeda I. **Pharmacokinetics of nalidixic acid and sodium nifurstyrenate in cultured fish following bolus intravascular administration**. Gyobyo Kenkyu 1996;31(4):191-6.
CBAC COPYRIGHT: CHEM ABS The present study examd. the pharmacokinetics of nalidixic acid (NA) in rainbow trout (Oncorhynchus mykiss) and sodium nifurstyrenate (NFS) in yellowtail (Seriola quinqueradiata) after a bolus intravascular administration. The doses of NA and NFS were 20mg/kg and 10mg/kg body wt., resp. Rainbow trout were kept in tanks with running fresh water at 15.0.+-.0.3.degree.C. Yellowtail were kept in tanks with running sea water at 21.3.+-.0.2.degree.C. Serunt concns. of NA and NFS were detd. using high performance liq. chromatog. Serum concns. of NA in rainbow trout and NFS in yellowtail were best described by a two-compartment open model. The half-lives for the distribution phase (T1/2alpha) and elimination phase (T1/2beta) were 1.4h and 13h for NA in rainbow trout and 0.6h and 7.7h for NFS in yellowtail, resp. The apparent vol. of distribution (Vd) and total body clearance (ClB) were estd. to be 1.01 l/kg and 54.7 mL/kg/h for NA in rainbow trout and 2.99l/kg and 271 mL/kg/h for NFS in yellowtail, resp. The serum protein bindings in vivo were detd. to be 10.9.+-.4.0% for NA in rainbow trout and 69.4.+-.7.5% for NFS in yellowtail, resp. As an application of this pharmacokinetic study, the oral bioavailability and the dosage regimens of NA in rainbow trout and NFS in yellowtail were briefly evaluated.

Uno K, Aoki T, Ueno R, Maeda I. **Pharmacokinetics of oxytetracycline in rainbow trout Oncorhynchus mykiss following bolus intravenous administration**. Fish Sci 1997;63(1):90-3.
CBAC COPYRIGHT: CHEM ABS The present study examd. the pharmacokinetics of oxytetracycline in rainbow trout Oncorhynchus mykiss. The pharmacokinetics of oxytetracycline were detd. after i.v. administration (50 mg/kg body wt.). Serum concns. of oxytetracycline were detd. using high performance liq. chromatog. with direct injection. Serum levels could be fitted to a two-compartment model. The calcd. half-lives for the distribution phase (T1/2alpha) and the elimination phase (T1/2beta) were 0.5 and 52 h, resp. The apparent vol. of distribution (Vdbeta) and total body clearance (ClB) were calcd. to be 1.46 l/kg and 19.6 mL/kg/h, resp. The area under the serum concn.-time curve was 2554 mug.cntdot.h/mL. Serum protein binding in vivo of oxytetracycline was 51.1.+-.7.4%. As an application of this pharmacokinetic study, the oral bioavailability and the dosage regimens of oxytetracycline in rainbow trout were briefly evaluated.

Van Der Molen GW, Kooijman S, Slob W. **A generic toxicokinetic model for persistent lipophilic compounds in humans: an application to TCDD**. Fundam Appl Toxicol 1996;31(1):83-94.
A physiologically based pharmacokinetic model of the life span kinetics of 2,3,7,8-tetrachloro-dibenzo-p-dioxin (1746016) (TCDD) in man was presented. The two unknown parameters were bioavailability and elimination rate. Blood flow rates were excluded, assuming an instantaneous distribution and long term equilibrium of TCDD among the model compartments. Each compartment was assumed to contain a fraction, dependent on relative organ size and lipid content, of the total body TCDD. Elimination of TCDD was taken to be a first order process proportional to liver tissue concentrations of TCDD. The TCDD concentration in each compartment was based on a constant amount of lipid in the compartment. The total concentration of TCDD was assumed to be total weight of TCDD in the body divided by the total body lipid weight. The six compartments of the model were blood, muscle, adipose tissue, bones, liver and remaining organs. Compartment weights were individually adjusted for the effects of age. Male and females were modeled separately. Intake rates, dependent on age, were calculated from the consumption of TCDD contaminated food by a cross section of the Dutch population in 1987. Comparison of the model with

literature data indicated that changes in environmental exposure and eating habits need to be considered. The authors conclude that the model is general in that it should describe the toxicokinetics of other lipophilic, slowly eliminated compounds.

Verhaar H, Morroni JR, Reardon KF, Hays SM, Gaver DJ, Carpenter RL, Yang R. **A proposed approach to study the toxicology of complex mixtures of petroleum products: the integrated use of QSAR, lumping analysis and PBPK/PD modeling**. Environ Health Perspect 1997;105(Suppl 1):179-95.
BIOSIS COPYRIGHT: BIOL ABS. Mixture toxicity is a topic that has become a matter of concern during the last two decades. One of the major problems with assessing the toxicity of mixtures and the associated human and environmental risk is the large number of possible mixtures, as well as the fact that the actual mixture effect for a given set of constituents might strongly depend on the actual composition of the mixture, i.e., the ratios of the constituent, as well as their nature. This paper presents a possible approach to describe and thereby better understand the pharmacokinetics and dynamics of complex mixtures by combining quantitative structure-activity relationships to predict needed parameters, lumping to reduce the complexity of the problem, and physiologically based pharmacokinetic/pharmacodynamic modeling to integrate all this information into a complete toxicological description of the mixture. It is our hope that by presenting this conceptual approach we might be able to stimulate some criticisms and discussions in the toxicology community regarding this complex and yet very important area of research.

Waller CL, Oprea TI, Chae K, Park HK, Korach KS, Laws SC, Wiese TE, Kelce WR, Gray LE Jr. **Ligand-based identification of environmental estrogens**. Chem Res Toxicol 1996;9(8):1240-8.

Yamauchi M, Ando M, Fujita K, Nagata Y. [**Activity change of nitric oxide dependent antioxidant enzymes. Glutathione peroxidase and catalase. In isolated rat superior cervical sympathetic ganglia evoked by carbachol stimulation during in vitro incubation**]. Fujita Gakuen Igakkaishi 1996;20(2):289-93. (Jpn)
CBAC COPYRIGHT: CHEM ABS Activation of glutathione peroxidase (GSH-PX) was obsd. on carbachol (muscarinic receptor agonist) stimulation of isolated rat superior cervical sympathetic ganglia (SCG). The carbachol-induced activation of GSH-PX was decreased in the presence of NO synthase inhibitor, L-NMMA. The carbachol-induced activation of GSH-PX was not affected by preganglionic denervation, but disappeared by postgangionic axotomy. These results are discussed in relation to the role of GSH-PX in the biodefense mechanism against carbachol-induced generation of reactive oxygen species.

Yee S, Choi BH. **Oxidative stress in neurotoxic effects of methylmercury poisoning**. Neurotoxicology 1996;17 (1):17-26.
The effects of methylmercuric chloride (MMC) on the rate of oxygen uptake were determined in purified cultures of oligodendrocytes, astrocytes, and cerebral cortical and cerebellar granular neurons obtained from embryonic and neonatal rat brains. Rapid and profound inhibition of oxygen uptake took place in all cell types following MMC exposure. However, the sensitivity of cellular respiration to the toxic effects of MMC appeared to parallel the normal oxygen demands of the cell type. To assess the effects of MMC on mitochondrial electron transport chain (ETC) activity, complex-specific electron donating substrates were used to stimulate the mitochondria obtained from both control and MMC-injected rat brains. Significant increases in reactive oxygen species (ROS) and thiobarbituric acid-reactive substances (TBARS), and a reduction in glutathione levels were observed in the MMC group following stimulation of complex III, but not with stimulation of either complex I or II, suggesting that MMC induces alterations in electron transport in the ubiquinol: cytochrome c oxidoreductase region. The rapidity of oxygen uptake inhibition by MMC in cultured CNS cells and the demonstration of MMC effects on specific enzyme complexes in the mitochondrial ETC strongly support the contention that mitochondria may be the earliest target of MeHg neurotoxicity, and that the mitochondrial ETC is the most likely site where excess ROS are generated in the brain to induce oxidative stress in MeHg poisoning.

Yu Z, Schwartz JB, Sugita ET, Foehl HC. **Five modified numerical deconvolution methods for**

biopharmaceutics and pharmacokinetics studies. Biopharm Drug Dispos 1996 Aug;17:521-40.
IPA COPYRIGHT: ASHP Four improved finite difference numerical deconvolution methods and 1 nonlinear regression numerical deconvolution method for biopharmaceutic and pharmacokinetic studies were developed and evaluated using simulated data generated with and without added noise under 6 different dosing cases. Comparisons between the methods were made in terms of the superimposability of calculated cumulative amount of drug released or absorbed-time profiles with the theoretical data. The fixed step number equal step length numerical deconvolution method was simple and accurate and would be appropriate for pharmacokinetic and biopharmaceutic studies. It was concluded that when an analytic function is legitimate to represent the drug input rate, the nonlinear regression numerical deconvolution method will yield enhanced numerical accuracy and stability.

Zelphati O, Szoka FC Jr. **Cationic liposomes as an oligonucleotide carrier: mechanism of action**. J Liposome Res 1997;7(1):31-49.
CBAC COPYRIGHT: CHEM ABS A review with 43 refs. Cationic liposomes are a useful in vitro but as yet unproven in vivo delivery system for oligonucleotides. An understanding of the mechanism of delivery mediated by cationic lipid/oligonucleotide complexes has been lacking. This review describes recent results concerning several steps of the delivery process, including the formation of complexes, the intracellular distribution of the oligonucleotide and the lipid, as well as the uptake pathway and site of intracellular release. Cationic liposomes form a polyelectrolyte complex with the oligonucleotides, protect them from nuclease degrdn., enhance their cellular uptake and improve the oligonucleotide potency. In the majority of cell types studied, cationic lipids deliver oligonucleotides into the cell predominately via an endocytotic pathway rather then by fusion with the plasma membrane. We propose that the oligonucleotide is released from the complex when anionic lipids from the cytoplasmic facing lipid monolayer of the cell flip into contact with the complex, the anionic lipids then laterally diffuse into the complex and form a charged neutralized ion-pair with the cationic lipids. This leads to displacement of the oligonucleotide from the cationic lipid and its release into the cytoplasm. In most cell types this occurs after endocytosis of the complex rather than after fusion of the complex directly with the plasma membrane. These new concepts of oligonucleotide release in cells provide a useful starting point for the rationale improvement of this nucleic acid delivery system.

Zhang YP, Macina OT, Rosenkranz HS, Karol MH, Mattison DR, Klopman G. **Prediction of the metabolism and toxicological profiles of gasoline oxygenates**. Inhalation Toxicol 1997;9(3):237-54.
CBAC COPYRIGHT: CHEM ABS Me tert-Bu ether (MTBE), Et tert-Bu ether (ETBE), tert-amyl Me ether (TAME), and diisopropyl ether (DIPE) were evaluated with CASE/MULTICASE structure relational models to det. their potential to pose human health risks. None of the parent ethers were predicted to be sensory irritants, eye irritants, contact sensitizers, mutagens, developmental toxicants, or carcinogens. The putative metabolites of ETBE were generated by META, an expert system, and evaluated for their potential to contribute to toxicity. Several of the metabolites were predicted by CASE/MULTICASE to be sensory irritants, contact sensitizers, mutagens, developmental toxicants, and carcinogens. A preliminary examn. of the putative metabolites of TAME and DIPE revealed the presence of epoxides, a class of chems. assocd. with developmental toxicity, carcinogenicity, and dermal contact sensitivity.

PULMONARY TOXICITY

[**Biotransformation and elimination of 1-nitropyrene in the isolated perfused lung: effects of pretreating rats with phenobarbitone, beta-naphthoflavone, benz(a)anthracene or their mixtures**]. Yaoxue Xuebao 1996;31 (8):568-76. (Chi)
CBAC COPYRIGHT: CHEM ABS The uptake, metab. and elimination of 1-nitropyrene (1-NP) by the isolated perfused lung (IPL) of rats pretreated with phenobarbitone (PB), beta-naphthoflavone (BNF), benz(a)anthracene (BA) or a mixt. of PB and BNF were studied. The IPL was perfused with 60 mL of recirculating Krebs-Ringer soln. contg. 150 mug of 1-NP for 1 h. The 1-NP was administered to the IPL by intratracheal or intravascular route. At

specific time points after 1-NP administration, perfusate samples were analyzed by HPLC. Monohydroxynitropyrenes, dihydroxynitropyrenes and 1-NP were in the perfusate. The time course of 1-NP concns. in the perfusate was described by a one-compartment pharmacokinetic model. Pretreatment with BNF, BA and/or PB + BNF decreased the mean residence time of 1-NP in perfusate and significantly enhanced the metab. of 1-NP and also the absorption of 1-NP, but PB had no effect on the pharmacokinetics of 1-NP.

Morris JB. **Uptake of acetaldehyde vapor and aldehyde dehydrogenase levels in the upper respiratory tracts of the mouse, rat, hamster, and guinea pig**. Fundam Appl Toxicol 1997;35(1):91-100.
Acetaldehyde is a ubiquitous indoor and outdoor air pollutant. This vapor is a respiratory tract irritant and a rodent inhalation carcinogen. The current study was aimed at examining the upper respiratory tract (URT) deposition efficiency for this vapor at inspired concentrations of 1, 10, 100, or 1000 ppm in four rodent species: B6C3F1 mouse, Sprague-Dawley rat, Syrian hamster, and Hartley guinea pig. For measurement of vapor uptake, the URT was isolated in urethane-anesthetized animals via insertion of a polyethylene cannula in the trachea such that its tip lay at the larynx. Uptake was measured under constant velocity unidirectional inspiratory flow at flow rates of approximately 50, 100, 200, and 300% of the predicted minute ventilation of each species and also under pseudo-cyclic flow (sinusoidal flow at 100% of the predicted minute ventilation with a constant 7 ml/min bleed for analysis). In addition, aldehyde dehydrogenase (AldDH) activities were measured in whole nasal tissue homogenates from each species for comparative purposes. In all species a high-affinity (Km < 0.2 MM), Low-capacity ALDDH isozyme was observed. In the mouse, hamster, and rat, a low-affinity (KM > 10 mM), high-capacity isozyme was observed; this isozyme was not observed in nasal tissue homogenates of the guinea pig. In all species, URT deposition efficiency was strongly dependent on the inspired concentration, with uptake being two- to three-fold more efficient at inspired concentrations of 1 or 10 ppm than at 1000 ppm. For example, at flows approximating the twice-minute ventilation rate URT uptake efficiency averaged 43, 49, 28, and 43% in the mouse, hamster, rat, and guinea pig, respectively, at an inspired concentration of 10 ppm, compared to 27, 14, 16, and 6% at an inspired concentration of 1000 ppm. Species differences were observed in uptake efficiency. At an inspired concentration of 1000 ppm a two-factor analysis of variance followed by a Newman-Keuls test revealed that uptake was significantly higher in the mouse, rat, and hamster than in the guinea pig. In contrast, at 10 ppm uptake was significantly lower in the rat than in any other species. Thus, the rank order of these species on the basis of ability to scrub acetaldehyde from the airstream differed at high compared to low inspired concentrations. The documentation of greatly differing deposition efficiencies as well as differing relative dosimetric relationships among species at high compared to low exposure concentrations highlights the potential complexities of quantitative extrapolation of high-concentration rodent inhalation toxicity data.

Thornton-Manning JR, Nikula KJ, Hotchkiss JA, Avila KJ, Rohrbacher KD, Ding X, Dahl AR. **Nasal cytochrome P450 2A: identification, regional localization, and metabolic activity toward hexamethylphosphoramide, a known nasal carcinogen**. Toxicol Appl Pharmacol 1997;142(1):22-30.
Two members of the cytochrome P450 2A subfamily, CYP2A10 and 2A11, are abundant nasal enzymes previously characterized in rabbit olfactory microsomes. Rabbit CYP2A is active toward a number of nasal toxicants, including the rat nasal procarcinogen hexamethylphosphoramide (HMPA). While P450s immunochemically related to the rabbit CYP2As have been detected in rat and human nasal mucosa, confirmation of these enzymes as members of the CYP2A subfamily and efforts to characterize their ability to bioactivate toxicants have been limited. In the present study, the regional distribution and cell-specific expression of CYP2A in the rat nasal cavity were examined using an antibody to rabbit CYP2A10/11. In sections of the anterior nose, immunoreactive CYP2A was present in ciliated cells of the nasal respiratory epithelium and cuboidal epithelial cells of the nasal transitional epithelium, but was absent in squamous epithelial cells. The most intense immunostaining was observed in the posterior nose. Olfactory sustentacular cells and Bowman's gland cells in sections posterior to the nasal papilla stained most intensely. Western blot analysis revealed that anti-CYP2A10/11 recognized a sharp band of approximately 50 kDa in nasal respiratory and olfactory microsomes, supporting the premise that the antibody is reacting with a cytochrome P450 enzyme. The nasal expression of CYP2A6 mRNA--a member of the human CYP2A subfamily having a high degree of homology to rabbit 2A10 and 2A11--was examined in human surgical patients. Middle turbinectomy tissues--largely composed of nasal respiratory epithelia--from 11 patients were analyzed for the

presence of CYP2A6 using reverse transcription-polymerase chain reaction (RT-PCR). Identification of CYP2A6 was confirmed by DNA sequencing of RT-PCR products. CYP2A6 mRNA was detected in all of the human samples analyzed. In additional experiments, human CYP2A6 metabolized HMPA to formaldehyde, suggesting that this compound might cause nasal toxicity in humans. The identification of CYP2A cytochromes in rat and human nasal tissues may have important implications for risk assessment of inhaled xenobiotics.

Zhuang ZX, Shen Y, Shen HM, Ng V, Ong CN. **DNA strand breaks and poly (ADP-ribose) polymerase activation induced by crystalline nickel subsulfide in MRC-5 lung fibroblast cells**. Hum Exp Toxicol 1996;15 (11):891-7.

QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIPS

Abou-Shaaban RR, Al-Khamees HA, Abou-Auda HS, Simonelli AP. **Atom level electrotopological state indexes in QSAR: designing and testing antithyroid agents**. Pharm Res 1996 Jan;13(1):129-36.
IPA COPYRIGHT: ASHP To develop antithyroid agents with reduced antioxidant effects, electrotopological state indexes of thiourylene moiety were used to synthesize 5 acyclic thiourylene-type drugs; the drugs were screened for antithyroid effects in rats after intraperitoneal administration, for antioxidant properties in vitro, and for acute toxicity in mice. Using the 125I-thiacyanate discharge technique, at least 1 compound showed potential value as an antithyroid agent. The relative efficacy of this compound after an equimolar dose was 102% and 51.5% of that of propylthiouracil with respect to the rate of 125I-discharge and 125I-uptake, respectively. In addition, chemiluminescence studies revealed that it has slight oxidant activity. It was concluded that the electrotopological state approach provides guidelines for designing new antithyroid drugs.

Abraham MH, Andonian-Haftvan J, Cometto-Muniz JE, Cain WS. **An analysis of nasal irritation thresholds using a new solvation equation**. Fundam Appl Toxicol 1996;31(1):71-6.
The development of a quantitative structure activity relationship (QSAR) equation for nasal pungency thresholds (NPTs) of 34 volatile organic compounds (VOCs) was reported. The equation was applied to literature values of NPTs measured in anosmic subjects. A two alternative, forced choice procedure with ascending concentration series was used to obtain NPT values. The selected VOCs included primary, secondary, and tertiary alcohols, n-aliphatic acetates, ketones, and other pungent VOCs. The Ferguson rule was applied to the set of VOCs. The solvation equation contained factors relating to electron pairs, dipolarity/polarizability, hydrogen bond acidity and basicity, and hydrophobicity. When descriptor values for the VOCs were applied in the equation, good agreement with reported NPTs was seen. The authors conclude that potency is determined by the efficiency of molecule transfer from air to the sensory biophase.

Amic D, Davidovic-Amic D, Beslo D, Lucic B, Trinajstic N. **The use of the ordered orthogonalized multivariate linear regression in a structure-activity study of coumarin and flavonoid derivatives as inhibitors of aldose reductase**. J Chem Inf Comput Sci 1997;37(3):581-6.
CBAC COPYRIGHT: CHEM ABS The relationship between mol. descriptors and the inhibitory activity of aldose reductase (AR) for a series of coumarin and flavonoid derivs. was investigated using a novel multivariate linear regression based on the ordered orthogonalized descriptor set. First, starting from the set of 31 descriptors, the authors produced absolutely the best nonorthogonalized QSAR models with I descriptors. These models are always better than the models that the most authors achieve by the use of the stepwise inclusion-exclusion procedure. In the next step, the authors realized all possible orthogonalization orderings of a given set of N descriptors (there are N] of these). The key result is that some orthogonalization orderings lead to QSAR models with I ordered orthogonalized descriptors that have higher values of both the correlation coeff. R and cross-validated correlation coeff. Rcv than the corresponding models with the same no. of nonorthogonalized descriptors. In order to achieve

the highest possible reliability in predicting the inhibition we produced several models that were obtained applying the ordered orthogonalization procedure on 1 set with 5 and on 2 sets with 7 descriptors. Then the inhibitory activity for 34 coumarins and 30 flavonoids was predicted, and several compds. were detected with a very high inhibitory activity.

Belvisi L, Bravi G, Catalano G, Mabilia M, Salimbeni A, Scolastico C. **A 3D QSAR CoMFA study of non-peptide angiotensin II receptor antagonists**. J Comput Aided Mol Des 1996;10(6):567-82.
CBAC COPYRIGHT: CHEM ABS A series of non-peptide angiotensin II receptor antagonists was investigated with the aim of developing a 3D QSAR model using comparative mol. field anal. descriptors and approaches. The main goals of the study were dictated by an interest in methodologies and an understanding of the binding requirements to the AT1 receptor. Consistency with the previously derived activity models was always checked to contemporarily test the validity of the various hypotheses. The specific conformations chosen for the study, the procedures invoked to superimpose all structures, the conditions employed to generate steric and electrostatic field values and the various PCA/PLS runs are discussed in detail. The effect of exptl. design techniques to select objects (mols.) and variables (descriptors) with respect to the predictive power of the QSAR models derived was esp. analyzed.

Bravi G, Gancia E, Mascagni P, Pegna M, Todeschini R, Zaliani A. **MS-WHIM, new 3D theoretical descriptors derived from molecular surface properties: a comparative 3D QSAR study in a series of steroids**. J Comput Aided Mol Des 1997;11(1):79-92.
CBAC COPYRIGHT: CHEM ABS The recently proposed WHIM (Weighted Holistic Invariant Mol.) approach has been applied to mol. surfaces to derive new 3D theor. descriptors, called MS-WHIM. To test their reliability, a 3D QSAR study has been performed on a series of steroids, comparing the MS-WHIM description to both the original WHIM indexes and CoMFA fields. The anal. of the statistical models obtained shows that MS-WHIM descriptors provide meaningful quant. structure-activity correlations. Thus, the results obtained agree well with those achieved using CoMFA fields. The concise no. of indexes, the ease of their calcn. and their invariance to the coordinate system make MS-WHIM an attractive tool for 3D QSAR studies.

Brzezinska E. **An application of TLC chromatographic data in QSAR assay of TIQs derivatives with beta2-adrenergic activity. Part II**. Acta Pol Pharm 1996;53(5):389-92.
CBAC COPYRIGHT: CHEM ABS The solns. of selected amino acids and TLC plates (silica-gel 60F254) were used for making of chromatog. models of beta-adrenergic interaction. A QSAR anal. of beta2-adrenergic activity and chromatog. data of 4,6,8-trihydroxy-, 6,7-dihydroxy- and 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline derivs. were made. A correlation between biol. activity data and behavior of the examd. compds. in chromatog. modifiable environment (M-1 - M-6) was investigated by linear regression anal. method.

Buolamwini JK, Raghavan K, Fesen MR, Pommier Y, Kohn KW, Weinstein JN. **Application of the electrotopological state index to QSAR analysis of flavone derivatives as HIV-1 integrase inhibitors**. Pharm Res 1996;13(12):1892-5.

Ciubotariu D, Muresan S, Gogonea V, Baleanu-Gogonea C, Medeleanu M, Ciubotariu C, Popescu M. **SDM - a new similarity/dissimilarity measure for QSAR studies**. Roum Biotechnol Lett 1997;2(2):114-30.
CBAC COPYRIGHT: CHEM ABS The computation of mol. similarity or dissimilarity of chem. bioactive compds. is a very important and fascinating research field for drug designers. Generally, mols. can be described in three distinct ways: (i) by their mol. graphs; (ii) by their atom position, and (iii) by their mol. fields. The quant. similarity measure can be developed for each of the above mol. characteristics. In this paper, we propose a new similarity/dissimilarity measure (SDM) developed on the basis of Minimal Steric Difference (MSD) method. Thus, the bioactive mols. are treated as hydrogen-depleted graphs and geometrical congruencies of std., S = :Si:i=1.m (i.e. the mol. of highest activity from the data base under study, whose shape is considered approx. complementary to the receptor cavity) vs. compared mol. X = :Xij:i=1.m j = 1,n are performed seeking the maximal superposition of two mols., small differences in bond lengths and bond angles being neglected. Because the steric parameter MSD is, in fact, the

Hamming distance, we transformed this particular dissimilarity function by normalization, similarly - but not identical - with the Rogers and Tanimoto procedure. In this way, the SDM parameter measure on the same scale both similarity and dissimilarity between mols. The range of variation is [0,1]: for SDM = 0, the degree of similarity is maximal and a value of 1 for SDM stands for maximal dissimilarity. The SDM parameter was applied with good results for QSAR study of inhibition of microsomal p-hydroxylation of aniline by alcs.: the correlation coeff. is r = 0.944 and the cross-validation coeff. is r2CV=0.736. The correlation between the logarithm of reciprocal concns. that causes 50% inhibition, i.e. pIC50 values, and SDM values for a series of 17 alcs. supports the hypothesis that the binding of alcs. to the enzymic site of cytochrome P 450 will proceed by a two stage mechanism, namely the Zipper mechanism.

Cooksey CJ, Land EJ, Rushton FA, Ramsden CA, Riley PA. **Tyrosinase-mediated cytotoxicity of 4-substituted phenols: use of QSAR to forecast reactivities of thiols towards the derived ortho-quinones**. Quant Struct Act Relat 1996;15(6):498-503.
CBAC COPYRIGHT: CHEM ABS Certain 4-substituted phenols can behave as tyrosine analogs and undergo tyrosinase-catalyzed oxidn. to cytotoxic o-quinones which react with crucial cellular thiols. Such phenols are potential melanogenesis-specific prodrugs for the chemotherapy of malignant melanoma. Previously (Cooksey et al. (1995) Anti-Cancer Drug Design 10, 119), by pulse radiolysis under oxidative conditions of the corresponding stable catechols in the presence and absence of thiols, we showed that the glutathione and cysteine reactivities of ten 4-substituted o-quinones correlated well with the Hammett sigmap consts. of the corresponding substituents. Starting from the corresponding catechols, one of which has not previously been described, we have now investigated six further 4-substituted o-quinones including two with substituent sigmap values higher than those previously studied. Extrapolation of the earlier quant. structure-activity relationships (QSAR) correctly predicted the high reactivities of these two o-quinones, with resp. substituents OCF3 and CH2NH3+, towards thiols. The data for all sixteen 4-substituted o-quinones, and for o-benzoquinone itself, have been combined to provide updated, statistically significant Hammett and Swain-Lupton correlations, including multivariate regressions using the data for both thiols. These relationships show that in reacting the substituted o-quinones with thiols, the rate consts. increase with the electron withdrawing capacities of the substituent groups, this being principally due to the resonance effect, with a smaller but significant contribution attributable to the field effect. Such QSAR may facilitate the design of improved melanogenesis-targeted anti-melanoma prodrugs.

Damborsky J, Schultz TW. **Comparison of the QSAR models for toxicity and biodegradability of anilines and phenols**. Chemosphere 1997;34(2):429-46.
Structure-activity models for toxicity and biodegradability of groups of m-anilines and p-phenols were developed and compared. Hydrophobicity was the most important property in determining toxicity. Whereas, electronic and steric properties were the more important in modeling biodegradation.

Devinsky F, Zamocka J, Lacko I, Polakovicova M. **QSAR and CAMM study of amphiphilic antimicrobially active 2,2'-bipyridyl monoammonium salts**. Pharmazie 1996 Oct;51:727-31.

Estrada E. **Spectral moments of the edge-adjacency matrix of molecular graphs. 2. Molecules containing heteroatoms and QSAR applications**. J Chem Inf Comput Sci 1997;37(2):320-8.

Gealy R, Graham C, Sussman NB, Macina OT, Rosenkranz HS, Karol MH. **Evaluating clinical case report data for SAR modeling of allergic contact dermatitis**. Hum Exp Toxicol 1996;15(6):489-93.
Clinical case reports can be important sources of information for alerting health professionals to the existence of possible health hazards. Isolated case reports, however, are weak evidence of causal relationships between exposure and disease because they do not provide an indication of the frequency of a particular exposure leading to a disease event. A database of chemicals causing allergic contact dermatitis (ACD) was compiled to discern structure-activity relationships. Clinical reports represented a considerable fraction of the data. Multiple Computer Automated Structure Evaluation (MultiCASE) was used to create a structure-activity model to be used in predicting

the ACD activity of untested chemicals. We examined how the predictive ability of the model was influenced by including the case report data in the model. In addition, the model was used to predict the activity of chemicals identified from clinical case reports. The following results were obtained: When chemicals which were identified as dermal sensitizers by only one or two case reports were included in the model, the specificity of the model was reduced. Less than one half of these chemicals were predicted to be active by the most highly evidenced model. These chemicals possessed substructures not previously encountered by any of the models. We conclude that chemicals classified as sensitizers based on isolated clinical case reports be excluded from our model of ACD. The approach described here for evaluating activity of chemicals based on sparse evidence should be considered for use with other endpoints of toxicity when data are correspondingly limited.

Hadjipavlou-Litina DJ. **QSAR studies of some sulfonamidobenzophenone oximes with antiviral activity**. J Pharm Pharmacol 1996;48(12):1215-7.

Hasegawa K, Miyashita Y, Funatsu K. **GA strategy for variable selection in QSAR studies: GA based PLS analysis of calcium channel antagonists**. J Chem Inf Comput Sci 1997;37(2):306-10.
CBAC COPYRIGHT: CHEM ABS The GAPLS (GA (genetic algorithm) based PLS (partial least squares)) program has been developed for variable selection in QSAR studies. The modified GA was employed to obtain a PLS model with high internal predictivity using a small no. of variables. To show the performance of GAPLS for variable selection, the program was applied to the inhibitory activity of dihydropyridine calcium channel antagonists. As a result, variables largely contributing to the inhibitory activity could be selected, and the structural requirements for the inhibitory activity could be estd. in an effective manner.

Heinisch G, Langer T, Lukavsky P. **Lipophilicity determination of diazine analogs of ridogrel. Part 2. Application of 3D QSAR for prediction of log k'w and log P**. Pharmazie 1996;51(11):840-2.
CBAC COPYRIGHT: CHEM ABS Log k'w and log P values of a series of 42 diazine analogs of the pyridine-derived thromboxane A2 synthetase inhibitor/receptor antagonist ridogrel were predicted using a 3D QSAR approach (CoMFA with the GRID H2O probe). The results were compared with the lipophilicity values established by anal. methods such as pH-metric titrn. for log D detn. and RP-HPLC for k'w calcn. For addnl. theor. prediction an increment method was employed. Whereas a good correlation was obsd. between log D or log k'w and log P values obtained by the increment method, the prediction method applying the 3D Qsar approach gave results which correlated excellently with the exptl. detd. ones.

Huang G, Sun H, Dai S. **Quantitative structure-activity relationship study for toxicity of organotin compounds on algae**. Bull Environ Contam Toxicol 1997;58(2):299-304.
CBAC COPYRIGHT: CHEM ABS Organotin compds. are a series of most extensively used organometallic compds. The inhibition effects of different kinds of organotins on the growth of two green algae, Scenedesmus obliquus and Platymonas sp., were studied in this lab. The toxicity mechanism of organotins was proposed based on the QSAR equations.

Isikdag I, Ucucu U, Buyukbingol E, Ozturk Y. **QSAR of smooth muscle relaxation by 2,5 - substituted benzimidazole derivatives**. Acta Pharm Turc 1997;39(1):1-6.
CBAC COPYRIGHT: CHEM ABS Quant. structure-activity analyses were carried out for in vitro relaxant actions of a series of 2,5-substituted benzimidazoles in rat duodenal smooth muscle. Analyses of simple and multiple linear regression models revealed modest to good correlations between the relaxant activity and structural properties of the benzimidazole derivs. such as steric, hydrophobic and electronic parameters. In the present study, it was obsd. that, as a collective property, parachor log(Par)+partition coeff. log(P) gives good results using either apparent affinity const. (pD2) or intrinsic activity (alphaE) as the predicted values. In this case, it was supposed that both the lipophilicity and the surface tension of mols. are responsible for the activity obsd.

Kamenska V, Nedyalkova Z, Invanov T, Mekenyan O. **Computer design and syntheses of antiulcer compounds. 2nd communication. N-substituted N'-[3-[3-(1-piperidinomethyl)phenoxy]propyl]ureas**. Arzneimforsch 1996;46(12):1144-8.
CBAC COPYRIGHT: CHEM ABS The in vitro and in vivo antiulcer effect of a series of N-substituted N'-[3-[3-(1-piperidinomethyl)phenoxy] propyl]ureas was modeled by making use the OASIS computer system for QSAR anal. Various research schemes were employed depending on structural representation of chems. under investigation, such as non-protonated (neutral), protonated at the piperidine and urea fragment nitrogens, and with intramol. hydrogen bonding. According to the modeling results, it is likely a variety of structural forms of antagonist mols. to take part in the receptor interaction. The QSAR study showed that the larger the electron acceptor properties of the nitrogen and oxygen atoms of the urea fragment, the higher is in vitro and in vivo activity of the antagonists.

Kim KH, Martin YC, Brooks CD. **Quantitative structure-activity relationships of 5-lipoxygenase inhibitors. Inhibitory potency of triazinone analogs in a broken cell**. Quant Struct Act Relat 1996;15(6):491-7.
CBAC COPYRIGHT: CHEM ABS Quant. structure-activity relationships (QSAR) of sixty 1-phenyl-[2H]-tetrahydrotriazin-3-one analogs are examd. for the inhibitory potency of 5-lipoxygenase in a broken cell, IC50. Potency is increased by lipophilic substituents at the 3'-, 5'-, and 5-positions. Potency is also increased with 3'- and 5'-substituents that withdraw electrons and decreased with 3',-substituents on the pyridyl ring that donate electrons. On the other hand, the potency decreases as the size of 2'-substituents increases. Thiourea analogs are about 1.6 times more potent than the corresponding carbonyl analogs.

Liao YY, Wang LS, He YB, Yang H. **Toxicity QSAR of substituted benzenes to yeast Saccharomyces cerevisiae**. Bull Environ Contam Toxicol 1996;56(3):460-6.

Lindgren F, Nouwen J, Loonen H, Worth A, Hansen B, Karcher W. **Environmental modeling based on a structural fragments approach**. Indoor Built Environ 1996;5(6):334-40.

Lindgren F, Sjostrom M, Berglind R, Nyberg B. **Modelling of the biological activity for a set of ceramic fibre materials: a QSAR study**. SAR QSAR Environ Res 1996;5(4):299-310.
The objective was to develop quantitative structure-activity relationships (QSARs) for a set of nine ceramic raw materials. The samples were characterized by a chemical analysis (both X-ray fluorescence and neutron activation analysis) and the morphology was determined by electron microscopy in combination with automated image analysis. Further, the fibre samples were subjected to two biological activity assays, measuring cytotoxicity and hydroxyl radical production. To investigate the produced data structures, principal component analysis (PCA) and partial least squares (PLS) were applied together with rigorous validation techniques. Significant QSARs were found for both biological activity assays. The morphology of the fibres plays an important role for the cytotoxicity and their trace element background is related to the hydroxyl radical production.

Lopez-Rodriguez ML, Rosado ML, Benhamu B, Morcillo MJ, Fernandez E, Schaper K. **Synthesis and structure-activity relationships of a new model of arylpiperazines. 2. Three-dimensional quantitative structure-activity relationships of hydantoin-phenylpiperazine**. J Med Chem 1997;40(11):1648-56.

Luan L, Zeng S, Liu Z, Fu X. [**Studies on the RP-HPLC retention behavior relationship between the structure of hydroxy compounds and their glucuronides**]. Gaodeng Xuexiao Huaxue Xuebao 1997;18(1):42-5. (Chi)
CBAC COPYRIGHT: CHEM ABS The quant. relation between the structure of solutes and their chromatog. retention behavior has been studied for many years. This paper discussed mainly the retention behavior of hydroxy compd. glucuronides. The samples were incubated in vitro. In conclusion, the retention behavior of glucuronides can be predicted by two ways: (1) prediction of capacity factor (k') for hydroxy compds. and their glucuronides is based on chem.-phys. consts.; (2) prediction of glucuronides capacity factor is based on the capacity factor of parent compd. The paper solved a knotty problem, the lack of glucuronide ref. by using biol. synthesis method. It is

very useful for the study on drug phase II metab. Predicting retention behavior of metabolites based on the parent compds. is also a new idea for studying QSAR for drug metabolites.

Luco JM, Ferretti FH. **QSAR based on multiple linear regression and PLS methods for the anti-HIV activity of a large group of HEPT derivatives**. J Chem Inf Comput Sci 1997;37(2);392-401.
CBAC COPYRIGHT: CHEM ABS Quant. structure-activity relationships have been developed for a set of 107 inhibitors of the HIV-1 reverse transcriptase, derivs. of a recently reported HIV-1 specific lead: 1-[(2-hydroxyethoxy) methyl]-6-(phenylthio)thymine (HEPT). The activity of these compds. was investigated by multiple linear regression (MLR) and PLS regression techniques and topol. indexes as well as several tabulated physicochem. substituent consts. were used as predictor variables. The results obtained indicate that the anti-HIV activity of the HEPT derivs. is strongly dependent on hydrophobic factors as expressed by the Hansch const. (.sum.pi(R1+R2)), and esp. dependent on the geometric factors mainly accounted for by the 1chiN(R2) and 4chipN mol. connectivity indexes and also for the mol. vol. (Vx), the Taft steric const. (Es(2R1)), and the Verloop parameter for the smallest width value (B1(3R1)). Besides, for this data set, comparison of the quality of MLR and PLS models show that PLS is a better approach to MLR for improving the interpretability of the data and also to exhibit models with a better predictive quality.

Mahmoudian M, Aghazadeh A. **QSARs of antifungal activity of furancarboxanilide derivatives against wild and mutant strains of Ustilago maydis**. J Sci Islamic Repub Iran 1997;8(1):18-22.
CBAC COPYRIGHT: CHEM ABS The structural requirements for the inhibitor activity of various furan carboxanilide derivs. against succinate dehydrogenase complex (SDC) activity in mitochondria of either wild or mutant strains of Ustilago maydis were investigated with the aid of Hansch QSAR anal. The inhibitor activity against both types of enzymes is best related to the SIGMAsigmaP or SIGMAsigmaM of the substituents on the Ph ring and to the hydrophobicity of substituents at ortho (with neg. slope) and meta position (with pos. slope). The activity decreases with the increase in the width of substituents at ortho position. However, while the activity decreases with the increase in the width of substituents at para position of Ph ring for the wild type enzyme, the reverse is true for the mutant type enzyme. In spite of wild type enzyme, the activity against mutant type enzyme is related to the presence of a hydrogen acceptor group at meta position of the Ph ring. The difference between wild and mutant types of SDC enzymes in the U. maydis has been brought about by the appearance of an amino acid residue with the ability to form hydrogen binding in the active site of the mutant enzyme.

McFarland JW, Berger CM, Froshauer SA, Hayashi SF, Hecker SJ, Jaynes BH, Jefson MR, Kamicker BJ, Lipinski CA, et al . **Quantitative structure-activity relationships among macrolide antibacterial agents: in vitro and in vivo potency against Pasteurella multocida**. J Med Chem 1997;40(9):1340-6.
CBAC COPYRIGHT: CHEM ABS Quant. structure-activity relationships were found among macrolide antibacterial agents in their potencies against the bacterial pathogen P. multocida both in vitro and in mouse infections. To obtain these relationships we measured, among other things, the pKa's and log P's of 15 known macrolides of diverse structures. Among these compds., in vitro potency [log(1/MIC)] is a function of log P, log D, and CMR (R = 0.86). In vivo potency is a function of the higher pKa, the HPLC chromatog. capacity factor log k', log(1/MIC) and pNF (R = 0.93). PNF is defined as the neg. logarithm of the fraction of neutral drug mols. present in aq. soln. at pH 7.4. The same phys. properties were detd. for 14 macrolides not used in developing the original QSAR models. Using the in vivo model, the authors calcd. the mouse protection potency ranges for these new compds. Ten ests. agreed with those obsd., 3 were lower by a half-order of magnitude, and one was calcd. to be active in the range of 15-50 mg/kg, but in fact was not active at 50 mg/kg, the highest level tested. When these new compds. were combined with the original 15, and the QSAR's updated, the new equations for the in vitro and in vivo potencies were essentially the same as those originally found. Hence, the phys. properties indicated above are major determinants of macrolide antibacterial potencies.

McKarns SC, Hansch C, Caldwell WS, Morgan WT, Moore SK, Doolittle DJ. **Correlation between hydrophobicity of short-chain aliphatic alcohols and their ability to alter plasma membrane integrity**. Fundam Appl Toxicol

1997;36(1):62-70.
The quantitative relationship between chemical structure and biological activity has received considerable attention in the fields of pharmacology and drug development. More recently, quantitative structure-activity relationships (QSARs) have been used for predicting chemical toxicity. It has been proposed that alcohols may elicit their toxic effects through hydrophobic interactions with the cellular membrane. The objective of this study was to evaluate the role of hydrophobicity in the loss of membrane integrity following acute exposure to short-chain aliphatic alcohols in rat liver epithelial cells in vitro. The series of alcohols studied included methanol, ethanol, 1-propanol, 1-butanol, 1-pentanol, 1-hexanol, 1-heptanol, 1-octanol, 2-butanol, 2-methyl-1-propanol, and 2-methyl-2-propanol. The lactate dehydrogenase (LDH) assay was used to quantify membrane integrity. The logarithm of the octanol/water partition coefficient (log P) was used to quantify hydrophobicity. LDH50 values, representing alcohol concentrations yielding a 50% increase in LDH release relative to untreated controls (i.e., mild disruption of membrane integrity), and EC50 values, representing alcohol concentrations yielding 50% of the maximal release of LDH (i.e., moderate disruption of LDH release), were experimentally determined for each alcohol. The LDH50 and EC50 values were then used to derive the QSAR relationship. The aqueous alcohol concentrations yielding LDH50 or EC50 values ranged from 8.9 x 10(-4) m (LDH50 for octanol) to 3.5 m (EC50 for methanol), and the log P of the alcohols ranged from -0.77 (methanol) to 3.00 (octanol). From these data, we have derived two QSAR equations describing the role of hydrophobicity in the release of LDH from rat liver epithelial cells following a 1-hr alcohol exposure. The QSAR equation for LDH50 values, log (1/LDH50) = 0.896 log P + 0.117 (n = 11, SD = 0.131), was nearly identical to the QSAR equation for EC50 values, log (1/EC50) = 0.893 log P + 0.101 (n = 11, SD = 0.133], suggesting that similar structure-activity relationships exist at both mild and moderate levels of membrane disruption. Our data indicate that an increase in LDH release was positively and linearly correlated with the hydrophobicity (r = 0.993). These data may help predict the potential biological effects of other, as yet untested, aliphatic alcohols and aliphatic alcohol-like compounds (e.g., anesthetics) on the plasma membrane.

Mekenyan OG, Bradbury SP, Kamenska VB. **Estimating one-electron reduction potentials of quinones**. SAR QSAR Environ Res 1996;5(4):255-68.
CBAC COPYRIGHT: CHEM ABS The one-electron redn. potential [E17] of benzo-, naphtho- and anthracenequinones is related to their ability to undergo redox cycling and elicit cytotoxicity through oxidative stress. To evaluate a general approach to est. the E17 of benzo-, naphtho- and anthracenequinones, QSAR approaches based on gas phase and solvation based methods were employed. Stereoelectronic descriptors of ground state quinones, resp. intermediates of the redox cycle, and the differences in parameters for the transition between intermediates were evaluated. The variation of E17 was correlated with descriptors of the parent quinones and specific transition parameters. The energy of the HOMO (the inverse of the ionization potential) and the energy of the LUMO of the parent benzoquinones were significantly correlated to E17. With the exclusion of ortho-hydroxy-substituted compds., the reaction enthalpies for the quinone-semiquinone couple, in combination with vol. polarizability, were significantly correlated to E17 across the entire dataset. The QSARs obtained were found to be consistent with the hypothesis that quinones which have a greater ability to delocalize electron d. should have more pos. redn. potentials for the quinone-semiquinone couple. In general, models incorporating solvation descriptors were found to be better correlated to E17 than those based on gas-phase descriptors, esp. when evaluated across structural classes of quinones.

Mekenyan OG, Veith GD, Call DJ, Ankley GT. **A QSAR evaluation of Ah receptor binding of halogenated aromatic xenobiotics**. Environ Health Perspect 1996;104(12):1302-10.
Because of their widespread occurrence and substantial biological activity, halogenated aromatic hydrocarbons such as polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs), and polychlorinated dibenzo-p-dioxins (PCDDs) comprise one of the more important classes of contaminants in the environment. Some chemicals in this class cause adverse biological effects after binding to an intracellular cytosolic protein called the aryl hydrocarbon receptor (AhR). Toxic responses such as thymic atrophy, weight loss, immunotoxicity, and acute lethality, as well as induction of cytochrome P4501A1, have been correlated with the relative affinity of PCBs, PCDFs, and PCDDs for the AhR. Therefore, an important step in predicting the effects of these chemicals is the estimation of their binding to the receptor. To date, however, the use of quantitative structure activity relationship

(QSAR) models to estimate binding affinity across multiple chemical classes has shown only modest success possibly due, in part, to a focus on minimum energy chemical structures as the active molecules. In this study, we evaluated the use of structural conformations other than those of minimum energy for the purpose of developing a model for AhR binding affinity that encompasses more of the halogenated aromatic chemicals known to interact with the receptor. Resultant QSAR models were robust, showing good utility across multiple classes of halogenated aromatic compounds.

Ortiz AR, Pastor M, Palomer A, Cruciani G, Gago F, Wade RC. **Reliability of comparative molecular field analysis models: effects of data scaling and variable selection using a set of human synovial fluid phospholipase A2 inhibitors**. J Med Chem 1997;40(7):1136-48.

Palluotto F, Carotti A, Casini G, Campagna F, Genchi G, Rizzo M, De Sarro GB. **Structure-activity relationships of 2-aryl-2,5-dihydropyridazino [4,3-b]indol-3(3H)-ones at the benzodiazepine receptor**. Bioorg Med Chem 1996;4(12):2091-104.
A large series of 2-aryl-2,5-dihydropyridazino[4,3-b]indol-3(3H)ones (PIs) carrying properly selected substituents at the indole and N2-phenyl rings was prepared and tested as central benzodiazepine receptor (BZR) ligands and potential (anti)convulsant agents. Stereoelectronic requirements for high receptor affinity were detected by means of 2-D and 3-D QSAR analyses. BZR affinities and pharmacological profiles of the compounds were examined in comparison with some other pyridazinoindolones recently described by us and with pyrazoloquinoline (PQ) analogues. An anticonvulsant activity greater than PQs was generally observed for PIs. Notably, in the test of audiogenically induced seizures, one compound showed a potency comparable to that of diazepam.

Pastor M, Cruciani G, Clementi S. **Smart region definition: a new way to improve the predictive ability and interpretability of three-dimensional quantitative structure-activity Relationship**. J Med Chem 1997;40 (10):1455-64.
CBAC COPYRIGHT: CHEM ABS This report describes a new methodol. aimed at grouping 3D-QSAR interaction energy descriptors into regions of neighbor variables bearing the same chem. and statistical information. These regions represent the structural variability of the series better than individual descriptor.

Patt WC, Edmunds JJ, Repine JT, Berryman KA, Reisdorph BR, Lee C, Plummer MS, Shahripour A, Haleen SJ, et al. **Structure-activity relationships in a series of orally active gamma-hydroxy butenolide endothelin antagonists**. J Med Chem 1997;40(7):1063-74.
CBAC COPYRIGHT: CHEM ABS The design of potent and selective non-peptide antagonists of endothelin-1 (ET-1) and its related isopeptides are important tools defining the role of ET in human diseases. In this report we will describe the detailed structure-activity relationship (SAR) studies that led to the discovery of a potent series of butenolide ETA selective antagonists. Starting from a micromolar screening hit, PD012527, use of Topliss decision tree anal. led to the discovery of the nanomolar ETA selective antagonist PD155080. Further structural modifications around the butenolide ring led directly to the subnanomolar ETA selective antagonist PD156707, IC50's = 0.3 (ETA) and 780 nM (ETB). This series of compds. exhibited functional activity exemplified by PD156707. This deriv. inhibited the ETA receptor mediated release of arachidonic acid from rabbit renal artery vascular smooth muscle cells with an IC50 = 1.1 nM and also inhibited the ET-1 induced contraction of rabbit femoral artery rings (ETA mediated) with a pA2 = 7.6.

Pham TT. [**Determination of antifungal activity of eugenol derivatives against C. albicans using the Hansch model**]. Hoa Hoc Cong Nghiep Hoa Chat 1996;(4):4-7. (Vie)
CBAC COPYRIGHT: CHEM ABS A QSAR math. model with substituent consts. was used for correlation anal. of the structure of eugenol derivs. with activity against C. albicans.

Pires JM, Floriano WB, Gaudio AC. **Extension of the frontier reactivity indexes to groups of atoms and**

application to quantitative structure-activity relationship studies. Theochem 1997;389(1-2):159-67.
CBAC COPYRIGHT: CHEM ABS The concept of frontier reactivity index, proposed by Fukui et al. (J. Chem. Phys., 20 (1952) 722), was extended to groups of atoms. The purpose of this procedure is to simulate frontier indexes capable of predicting the susceptibility of a chem. group to an external electronic interaction, such as nucleophilic, electrophilic or radical. The group frontier indexes can be useful in quant. structure-activity relation (QSAR) studies, where substituent consts. for groups of atoms are widely used. The group frontier indexes are obtained through the sum of the corresponding at. frontier indexes. The at. frontier indexes were computed from AM1 wavefunction coeffs. The frontier indexes considered in this article, i.e. frontier electron, orbital and radical densities, are shown to be relatively independent of the mol. geometry. We applied the group frontier index concept to a QSAR study of triazene derivs. The group frontier indexes that appear to be significant in the correlation anal. are consistent with the known hydroxylation mechanism of triazene induced mutagenesis.

Raj HG, Gupta S, Biswas G, Singh S, Singh A, Jha A, Bisht KS, Sharma SK, Jain SC, Parmar VS. **Chemoprevention of carcinogen-DNA binding: the relative role of different oxygenated substituents on 4-methylcoumarins in the inhibition of aflatoxin B1-DNA binding in vitro**. Bioorg Med Chem 1996;4(12):2225-8.
Eighteen 4-methylcoumarins bearing methoxy/hydroxy/acetoxy functionalities have been reported to effectively inhibit the rat liver microsome-mediated aflatoxin B1-DNA binding in vitro. The contribution of functionality on coumarin nucleus towards the inhibition of AFB1-DNA binding is in the order acetoxy > hydroxy > methoxy. The results illustrate the structure-activity relationship.

Schnitker J, Gopalaswamy R, Crippen GM. O**bjective models for steroid binding sites of human globulins**. J Comput Aided Mol Des 1997;11(1):93-110.
CBAC COPYRIGHT: CHEM ABS We report the application of a recently developed alignment-free 3D QSAR method [Crippen, G. M., (1995)] to a benchmark-type problem. The test system involves the binding of 31 steroid compds. to two kinds of human carrier protein. The method used not only allows for arbitrary binding modes, but also avoids the problems of traditional least-squares techniques with regard to the implicit neglect of informative outlying data points. It is seen that models of considerable predictive power can be obtained even with a very vague binding site description. Underlining a systematic, but usually ignored problem of the QSAR approach, there is not one unique type of model but, rather, an entire manifold of distinctly different models that are all compatible with the exptl. information. For a given model, there is also a considerable variation in the found binding modes, illustrating the problems that are inherent in the need for 'correct' mol. alignment in conventional 3D QSAR methods.

Schultz TW, Cronin MT. **Quantitative structure-activity relationships for weak acid respiratory uncouplers to Vibrio fisheri**. Environ Toxicol Chem 1997;16(2):357-60.
BIOSIS COPYRIGHT: BIOL ABS. Acute toxicity values (5- and 30-min Vibrio fisheri 50% luminescence inhibition) of 16 organic compounds thought to elicit their response via the weak acid respiratory uncoupling mechanism of toxic action were secured from the literature. Regression analysis of toxicities revealed that a measured 5-min V. fisheri potency value can be used as a surrogate for the 30-min value. Regression analysis of toxicity (30-min for potency (log pT30-1)) versus hydrophobicity, measured as the 1-octanol/water partition coefficient (log Kow), was used to formulate a quantitative structure-activity relationship (QSAR). The equation log pT30-1 = 0.489(log Kow) + 0.126 was found to be a highly predictive model (r2 adj. = 0.848). This V. fisheri QSAR is statistically similar to QSARs generated from weak acid uncoupler potency data for Pimephales promelas survivability and Tetrahymena pyriformis population growth impairment. This work, therefore, suggests that the weak acid respiratory uncoupling mechanism of toxic action is present in V. fisheri, and as such is not restricted to mitochondria-containing organisms.

Sharma V, Goswami R, Madan AK. **Eccentric connectivity index: a novel highly discriminating topological descriptor for structure-property and structure-activity studies**. J Chem Inf Comput Sci 1997;37(2):273-82.
CBAC COPYRIGHT: CHEM ABS A novel, distance-cum-adjacency topol. descriptor, termed as eccentric connectivity index, has been conceptualized, and its discriminating power has been investigated with regard to

phys./biol. properties of mols. Correlation coeffs. ranging from 95% to 99% were obtained using eccentric connectivity index in various datasets with regard to phys. properties of diverse nature. These correlations were far superior to those correspondingly derived from the Wiener index. For structure-activity studies, a dataset, comprised of 94 substituted piperidinyl Me ester and methylene Me ester analogs as analgesic agents, was selected. Values of the eccentric connectivity index, the Wiener index, and Randic's mol. connectivity index were calcd., and active ranges were identified. Good correlations between topol. descriptors and analgesic activity of these analogs were obtained. Eccentric connectivity index exhibited highest predictability of the order of 86%. High discriminating power as revealed by excellent correlations obtained from structure-property and structure-activity studies offers an eccentric connectivity index of vast potential in QSPR/QSAR.

Shelley ML, Harris RL, Boehlecke BA. **A mathematical model of bronchial absorption of vapors in the human lung and its significance in pharmacokinetic modeling**. SAR QSAR Environ Res 1996;5(4):221-53.
CBAC COPYRIGHT: CHEM ABS This work formulates a model description of moderately sol. gas and vapor uptake in the bronchial region of the lung during initial inhalation exposure. The mass transport problem is solved using an iterative-anal. approach in which inhaled chem. is partitioned to alveolar blood-gas exchange, bronchial wall absorption, and exhalation. Results of 109 simulations allowed regression anal. to provide simple algebraic equations to describe the fraction of total inhaled mass taken up by the two alternative absorption pathways. These fractions are dependent on the chem.'s soly. and liq. diffusivity. The derived relationships are then used in a modified physiol. based pharmacokinetic (PBPK) model which accounts for bronchial uptake. The results are compared to identical results from a traditional (unmodified) PBPK model to draw conclusions concerning the effect of bronchial uptake on systemic chem. distribution in addn. to elevated bronchial tissue.

Trinajstiv N, Nikolic S, Lucic B, Amic D. **On QSAR modeling**. Acta Pharm Zagreb 1996;46(4):249-63.
CBAC COPYRIGHT: CHEM ABS The complexity of living matter is enormous. To understand the interaction between a drug and an organism is a very difficult, if not impossible, task. Modeling of some kind is, therefore, essential in drug research. One approach to model the activity of a drug is by quant. structure-activity relation (QSAR). The QSAR models are based on the empirical observation that there exists a connection between the structure of a drug and its biol. response. QSAR models are ordinarily created for sets of structurally related mols. whose activities are quant. recorded. This demands that the structure of a mol. must also be numerically encoded. Since mols. are discrete objects, this is not a simple undertaking. Approaches to encode the structure of mols. numerically are presented. The two sets of nos. (activities and nos. representing mol. structures) are then statistically analyzed. Thus, the most often used QSAR models are regression models. A classification of QSAR models, based on alternatives that have been used to encode numerically the mol. structure, is given. An original approach to construction of QSAR models via ordered orthogonalized descriptors and its application is described. The procedure is used to predict the inhibition of cAMP phosphodiesterase by flavone derivs.

Ungwitayatorn J, Pickert M, Frahm AW. **Quantitative structure-activity relationship (QSAR) study of polyhydroxyxanthones**. Pharm Acta Helv 1997;72(1):23-9.

Xue F, Wang H, Zhao R. [**Quantitative structure activity relationship of the aromatic carboxylic acid derivatives**]. Zhongguo Shouyi Xuebao 1996;16(4):378-82. (Chi)
CBAC COPYRIGHT: CHEM ABS The repellency and QSAR of forty arom. carboxylic acid derivs. were analyzed using Hansch's method in the study. The results indicated that the repellency of a series of the compds. is significantly correlated with the boiling coeff. The size of substituent of the compds. should not be larger than benzene, the most appropriate value of the partition coeff. (logP) is about 3.50.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Conway G, Margoliath A, Wong-Madden S, Roberts RJ, Gilbert W. **Jak1 kinase is required for cell migrations and anterior specification in zebrafish embryos**. Proc Natl Acad Sci U S A 1997;94(7):3082-7.
Establishment of the vertebrate body plan requires a variety of signaling molecules. In a search for tyrosine kinases expressed in early zebrafish embryos, a model system for the study of vertebrate development, we discovered Jak1 kinase to be maternally encoded and the mRNA evenly distributed among the cells of blastula-stage embryos. Injection of RNA-encoding dominant-negative Jak1 kinases reduces a specific cell migration, epiboly, and results in the reduction of goosecoid expression and of anterior structures. This work establishes that, in addition to its role in signal transduction of cytokines in adult tissues, Jak1 kinase has a role in early vertebrate development.

Elmazar M, Nau H. **Toward the mechanism of valproate teratogenesis: structural basis and interactions with agents that alter folate metabolism**. Saudi Pharm J 1997;5(1):6-16.
CBAC COPYRIGHT: CHEM ABS The hypothesis that valproate-induced neural tube defects (NTDs) may be due to interference with folate metabolic pathways was investigated by studying the possible interactions of valproate and a no. of agents that modulate folate metab. Valproate (VPA)-induced exencephaly (an anterior NTDs) in NMRI mice was used as an animal model. A single dose of valproic acid sodium salt (300-500 mg/kg, s.c.) on day 8 of gestation produced a dose related increase in exencephaly rate, embryolethality, and fetal wt. retardation. Supplementation with vitamin B6+B12 without and with folinic acid, serine with folinic acid, and carnitine was found to reduce valproate-induced exencephaly rate. Vitamin B6+B12 and methionine reduced VPA-induced fetal wt. retardation and embryotoxicity, resp. The protection was not complete and was not always dose related, and in case of carnitine, higher doses were devoid of such effects and even increased valproate-induced exencephaly. Coadministration of valproate with low (threshold) doses of methotrexate, trimethoprim, nitrous oxide and ethanol was found to increase the incidence of exencephaly rate. Embryotoxicity was also increased as a result of such combinations except with trimethoprim. The obsd. effects were not due to altered valproate toxicokinetics in case of methotrexate and trimethoprim but was probably due to decreased valproate elimination by ethanol and advise against the use of these agents in valproate-treated epileptics during pregnancy. The previous results support the view that valproate-induced NTDs may be mediated via an interaction with folate metab. Study of the structural-activity relationships of several valproate analogs revealed a strict structural requirement for high teratogenic potency. In contrast, the anticonvulsant activity and neurotoxicity showed broader structural specificity. Furthermore, the R- and S-enantiomers of 2-n-propyl-4-pentenoic acid and 2-n-propyl-4-pentynoic acid showed different teratogenic activity (S-enantiomers were more teratogenic than R-enantiomers) in contrast to anticonvulsant potency in the absence of pharmacokinetic differences. These findings opens the possibility for development of novel antiepileptic agents with low teratogenic potency.

Fahraeus-Van REE Ge, Payne JF. **Effect of toxaphene on reproduction of fish**. Chemosphere 1997;34(4):855-67.
BIOSIS COPYRIGHT: BIOL ABS. The effect of Toxaphene on fish reproduction was investigated in sexually mature female zebrafish fed for two weeks with food contaminated with three different concentrations of the Pesticide (0.02, 0.23 and 2.2 mug/g fish/day). No overt differences were observed in reproductive success as assessed by examination of (a) total number of eggs spawned by each female, (b) percentage of fertilized eggs 24 hours after fertilization, (c) percentage of embryo mortality 72 hours after fertilization and (d) percentage of hatching 72 hours after fertilization. By contrast Toxaphene produced distinct effects of a dose response nature on oviposition. This observation is not only of interest with respect to Toxaphene, but also points to the importance of evaluating other pesticides for their effect on the oviposition of feral species that might be even more sensitive than zebrafish. Toxicity of Toxaphene was manifested both in the parent fish (skin discoloration, subcutaneous hemorrhages, particularly around the gill areas and backbones curved in the vertical plane) and in hatching embryos (half hatched).

Fort DJ, Stover EL. **Effect of low-level copper and pentachlorophenol exposure on various early life stages of Xenopus laevis**. ASTM Spec Tech Publ 1996;1306:188-203.
CBAC COPYRIGHT: CHEM ABS An evaluation of the effects of low-level copper and pentachlorophenol exposure

on various early life stages of the South African clawed frog, X. laevis, was performed using stage-specific and long-term continuous exposures. Stage-specific exposure expts. were conducted such that sep. subsets of embryos and larvae from the same clutch were exposed to 2 toxicants, copper and pentachlorophenol, from 0 d to 4 d (std. Frog Embryo Teratogenesis assay-Xenopus [FETAX]), 4 d to 8 d, 8 d to 12 d, and 12 d to 16 d. Results from 2 sep. concn.-response expts. indicated that sensitivity to either toxicant increased in each successive time period. Longer-term exposure studies conducted for 60 to 75 days indicated that copper, but not pentachlorophenol, induced redn. deficiency malformations of the hind limb at concns. as low as 0.05 mg/L. Pentachlorophenol concns. as low as 0.5 mug/L inhibited tail resorption. However, copper did not adversely affect the process of tail resorption. These results indicated that studies evaluating longer-term developmental processes are important in ecol. hazard evaluation.

Gaffield W, Keeler RF. **Steroidal alkaloid teratogens: molecular probes for investigation of craniofacial malformations**. J Toxicol Toxin Rev 1996;15(4):303-26.

Henry TR, Spitsbergen JM, Hornung MW, Abnet CC, Peterson RE. **Early life stage toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in zebrafish (Danio rerio)**. Toxicol Appl Pharmacol 1997;142(1):56-68.
BIOSIS COPYRIGHT: BIOL ABS. Toxicity and histopathology of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in zebrafish (Danio rerio) early life stages was characterized from 12 to 240 hr postfertilization (hpf) following waterborne exposure of newly fertilized eggs. TCDD did not increase egg mortality (0-48 hpf), nor did it affect time to hatching (4896 hpf). Egg doses of 1.5 ng (3H)TCDD/g or greater elicited toxic responses in zebrafish larvae. Pericardial edema and craniofacial malformations were first observed at 72 hpf, followed by the onset of yolk sac edema (96 hpf) and mortality (132 hpf). At 240 hpf the ED50s for pericardial edema, yolk sac edema, and craniofacial malformations were 2.2, 2.1, and 1.9 ng (3H)TCDD/g egg, respectively. The LD50, determined at 240 hpf, was 2.5 ng (3H)TCDD/g egg. Severe hemodynamic changes, observed as slowed blood flow in vascular beds of the trunk, head, and gills and slowed heart rate, occurred in TCDD-treated zebrafish prior to or coincident with.

Hughes CM, Lewis SE, McKelvey-Martin VJ, Thompson W. **Reproducibility of human sperm DNA measurements using the alkaline single cell gel electrophoresis assay**. Mutat Res 1997;374(2):261-8.
CBAC COPYRIGHT: CHEM ABS The single cell gel electrophoresis (SCGE) assay is a simple visual technique used to assess DNA integrity in individual cells by measuring damage reflected as strand breaks under alk. conditions. Cells are embedded in agarose on glass slides followed by lysis of the cell membranes after which damaged DNA strands are electrophoresed away from the nucleus towards the anode and deposited to one side giving the appearance of a tail. DNA damage may be measured by assessing the relative amts. of DNA remaining in the head as opposed to those strands which have formed the tail. The assay has been used to det. DNA quality in human sperm (Hughes, C.M., S.E.M. Lewis, V.J. McKelvey-Martin, W. Thompson, A comparison of baseline and induced DNA damage in human sperm from fertile and infertile man, using a modified comet assay. Mol. Human Reprod., in press) by measuring fifty cells on one slide for each individual. Coeffs. of variation between three control slides prepd. for ten individuals were less than 4% and less than 9% for three slides prepd. using irradiated sperm. Ten readings of fifty sperm each from a single slide showed a coeff. of variation of less than 6% for ten individuals studied. These results indicate that the measurement of fifty sperm from a single slide is sufficient to assess the DNA damage within a sperm population. Coeffs. of variation of less than 5.4% for repeated anal. of individual cells were obtained which demonstrates the reproducibility of the image anal. software.

Illanes J, Blanquez MJ, Gonzalez ME, Rojo C. [**The teratogenic effect of alcohol on chicken embryos**]. Anat Histol Embryol 1995;24(4):217-21. (Spa)
BIOSIS COPYRIGHT: BIOL ABS. The teratogenic effect of alcohol on chick embryos has been confirmed by many investigators. However, how this occurs is unknown. The aim of this study was to establish a teratogenic pattern of alcohol effects, on the first stages of development in avians. Fertilized eggs were infused through the air space of the shell on day 0, with ethanol in concentrations of 20%, 40% and 60%. The control group was infused with 0.1 ml of NaCl at 0.9%. At a second stage, the eggs were treated on the 4th day of incubation, using the same method. In both groups the eggs were removed on the 11th day of incubation. The teratological manifestations that appeared

more frequently were evisceration, haemorrhagic embryos, oedema, cranial deformities, lack of eyes, and umbilical hernia, showing every embryo a clear reduction in size and body weight.

Paragas VB, Zhang Y, Haugland RP, Singer VL. **The ELF-97 alkaline phosphatase substrate provides a bright, photostable, fluorescent signal amplification method for FISH**. J Histochem Cytochem 1997;45(3):345-57.
CBAC COPYRIGHT: CHEM ABS We used the ELF-97 (Enzyme-Labeled Fluorescence) phosphatase substrate, 2-(5'-chloro-2-phosphoryloxyphenyl)-6-chloro-4(3H)-quinazolinone, with alk. phosphatase conjugates of streptavidin and appropriate antibodies to amplify signals from biotinylated and haptenylated hybridization probes. The dephosphorylated product, ELF-97 alc., is a bright yellow-green fluorescent ppt. optimally excited at .apprx.360 nm, with emission centered at .apprx.530 nm. This large Stokes shift allows ELF-97 signals to be easily distinguished from sample autofluorescence and signals arising from counterstains or other fluorophores. The ELF-97 ppt. was extremely photostable compared to fluorescein, allowing multiple photog. exposures of samples without significant signal intensity loss. For RNA in situ hybridization, labeling was specific and localized well to targets in cultured cells, tissue sections, and whole-mount zebrafish embryos. ELF-97 signals developed in seconds to minutes and were easily distinguished from pigmented tissues or cells, unlike those obtained using colorimetric substrates. We used the substrate with singly biotinylated short oligonucleotides to detect actin mRNA in MDCK cells and actin and beta-galactosidase mRNA in LacZ+ mouse fibroblasts. We also used a biotinylated cDNA, complementary to the mRNA encoded by the const. region of the T-cell receptor beta-chain, to specifically identify T-cells in mouse lymph node tissue sections. With digoxigenin-labeled probes, we detected several developmentally expressed mRNAs in whole-mount zebrafish embryos. Hybridization to centromere repeat regions in human metaphase and interphase chromosomes was also detected; ELF-97 signals were manyfold brighter than signals obtained with fluorescein conjugates. Finally, Southern blot hybridization using singly labeled oligonucleotide probes yielded a sensitivity similar to that obtained with radioactivity.

Patel VD, Nolan RS, Hu N, Clark EB, Hootnick DR, Levinsohn EM, Packard D S Jr. **Embryonic hypertension following exposure to teratogenic doses of 5-fluoro-2'-deoxyuridine**. Biomed Environ Sci 1996;9(4):408-17.
BIOSIS COPYRIGHT: BIOL ABS. The teratogenicity of 5-fluoro-2'-deoxyuridine (FdU) is well established. Previously, we have demonstrated that teratogenic doses of FdU produce hematomas and suggested that those hematomas produced skeletal malformations in chicken embryos. In this study, the cardiovascular effects of teratogenic doses of FdU in chicken embryos were studied. A dose of either 0.026 mug FdU or 0.030 mug FdU was injected into the yolk sacs of fertile chicken eggs containing embryos at Hamburger and Hamilton stages 17-19 of development. The embryos were then returned to the incubator. Aortic systolic and diastolic blood pressure, blood velocity and heart rate were measured at stages 21, 24 or 27 using a servonull system and Doppler ultrasound. In addition, mean arterial blood pressure, blood flow, and stroke volume were calculated from these data. Similar data were also recorded from uninjected and saline injected control embryos. Systolic and mean arterial blood pressures were significantly increased in FdU-treated embryos at stage 27. The other parameters measured or calculated were not significantly different from control embryos. Our study suggests that elevated systolic blood pressure in chicken embryos treated with FdU may lead to hematoma formation and subsequent birth defects.

Petkovich M, Ohno C, Jones B, inventors, Queen's University at Kingston a. **Zebrafish retinoid receptors RXRdelta and RXRepsilon, cDNA sequences, heterodimers with steroid receptor, and transgenic expression in mammal cell**. Canadian Patent Appl Patent 2,177,642. 1996 Jun 12
CBAC COPYRIGHT: CHEM ABS Zebrafish retinoic acid receptors RXRdelta and RXRepsilon are disclosed. These two receptors are members of the retinoic X receptor (RXR) family of nuclear receptors. These receptors exhibit a high degree of amino acid conservation with other vertebrate RXRs, but contain a conserved sequence which encodes an addnl. fourteen amino acid segment within their ligand binding domains. These RXRs do not bind 9-cis retinoic acid (RA) or all-trans RA with high affinity and are not activated by 9-cis RA. These RXR's are able to form dimers in a manner equiv. to that of other RXR's. Mammal cell line CV-1 was transformed to express zebrafish RXR receptor. Heterodimer of type II steroid hormone receptor and zebrafish RXR receptor binds DNA.

Saillenfait AM, Langonne I, Sabate JP. **Developmental toxicity of trichloroethylene, tetrachloroethylene and four of their metabolites in rat whole embryo culture**. Arch Toxicol 1995;70(2):71-82.
BIOSIS COPYRIGHT: BIOL ABS. The embryotoxicity of trichloroethylene (TRI), tetrachloroethylene (PER), and of four of their oxidative metabolites i.e. trichloroacetic acid, dichloroacetic acid, chloral hydrate, and trichloroacetyl chloride, was studied in vitro, using the rat whole embryo culture system. Embryos from Sprague-Dawley rats were explanted on gestational day 10 (plug day 0) and cultured for 46 h in the presence of the test chemical. All of the tested chemicals produced concentration-dependent decreases in growth and differentiation and increases in the incidence of morphologically abnormal embryos. TRI and PER produced qualitatively similar patterns of abnormalities, while TRI and/or PER metabolites, each elicited clearly distinguishable dysmorphogenic profiles. The presence of hepatic microsomal fractions in the culture medium produced marked decreases in TRI- and PER-induced embryotoxic effects, including mortality, severity of malformations, and delayed growth and differentiation.

Takahashi M. [**Detection of DNA injury in early embryo by using the comet assay method**]. Brain Techno News 1996;58:16-8. (Jpn)
CBAC COPYRIGHT: CHEM ABS DNA injury of single cell by culture environment was found to be detected by comet assay (micro cell gel electrophoresis). Single cell was embedded into agarose gel on a slide glass and the protein components of the cell were solubilized and removed by the treatment in alk. soln. contg. surface-active agent, and the exposed DNA was applied to gel electrophoresis. The method was applied to the embryo developed in vivo and the embryo cultured in vitro. As a result, the DNA injury of the cell cultured in vitro was demonstrated by the method

Troxel CM, Reddy AP, O'Neal PE, Hendricks JD, Bailey GS. **In vivo aflatoxin B1 metabolism and hepatic DNA adduction in zebrafish (Danio rerio)**. Toxicol Appl Pharmacol 1997;143(1):213-20.
The zebrafish (Danio rerio) is assuming prominence in developmental genetics research. By comparison, little is known of tumorigenesis and nothing is known of carcinogen metabolism in this species. This study evaluated the ability of zebrafish to metabolize a well-characterized human carcinogen, aflatoxin B1 (AFB1), to phase I and phase II metabolites and assessed hepatic AFB1-DNA adduction in vivo. Fish i.p. injected with 50-400 micrograms [3H] AFB1/kg body wt displayed a linear dose response for hepatic DNA binding at 24 hr. AFB1-DNA adduct levels among treatments showed no statistical difference over the period from 1 to 21 days after injection, suggesting poor adduct repair in this species. DNA binding in female fish was 1-7-fold higher than that in males ($p < 0.01$). An in vitro AFB1 metabolism assay verified that Zebrafish liver extracts oxidize AFB1 to the 8,9-epoxide proximate electrophile (KM = 79.0 +/- 16.4 Microm, VMAX = 11.7 +/- 1.4 PMOL/MIN/MG protein at 28 degrees C). The excretion of AFB1 and its metabolites was also examined by HPLC. As is typical of other fish studied, major metabolites excreted were aflatoxicol (AFL) and Aflatoxicol-glucuronide (AFL-G), followed by unreacted AFB1. AFL appeared as early as 5 min after injection, whereas AFL-G was a significant metabolite after 18 hr.This study shows that in vivo administration of AFB1 to zebrafish results in moderate addcution of the carcinogen to Liver DNA and that zebrafish have the capacity for both phase I and phase II metabolism of AFB1. The approximate fourfold difference between rainbow trout and zebrafish AFB1-DNA covalent binding index appears insufficient to explain the relative resistence of zebrafish to dietary AFB1 hepatocarcinogenicity.

Willett CE, Zapata AG, Hopkins N, Steiner LA. **Expression of zebrafish rag genes during early development identifies the thymus**. Dev Biol 1997;182(2):331-41.
Recent experiments have demonstrated that zebrafish is a vertebrate in which it is possible to carry out large-scale mutagenic screens to identify genes involved in specific developmental pathways. To follow development of the immune system in zebrafish, we have analyzed the expression of the recombination activating genes, rag1 and rag2, which we have previously isolated and characterized. These genes catalyze the rearrangement of immunoglobulin genes in immature B lymphocytes and of T cell receptor genes in immature T lymphocytes and are therefore appropriate markers to follow the development of organs.

Wubah JA, Ibrahim MM, Gao X, Nguyen D, Pisano MM, Knudsen TB. **Teratogen-induced eye defects mediated**

by p53-dependent apoptosis. Curr Biol 1996;6(1):60-9.
BIOSIS COPYRIGHT: BIOL ABS. Background: Many birth defects are believed to involve gene-environment interactions, although the mechanisms involved are poorly understood. Apoptosis is a common effect of many kinds of environmental stresses on the developing embryo; therefore, mechanisms of teratogenesis may be approached within the context of the cell death program. The p53 tumor suppressor gene encodes a transcription factor which functions as a critical regulator of apoptosis in response to environmental stress. Results: To investigate the relationship between p53-dependent apoptosis and teratogenesis, we subjected day 8 mouse embryos with different p53 gene backgrounds to a genotoxic stress, 2-chloro-2'-deoxyadenosine. Treatment rapidly stimulated nuclear p53 accumulation and triggered apoptosis in some (head-fold) but not other (primitive heart) developing structures. Induced cell death was p53 gene-dose dependent, as shown by the intermediate sensitivity of 4-5 somite stage embryos bearing only a single effective p53 allele and the lack of sensitivity of p53-null mutants. Abnormal development was manifested as eye defects by day 11, particularly lens agenesis. Overall the incidences of these defects at term were 73.3% for p53 wild-type fetuses, 52.5 % for heterozygous mutants, and 2.2% for p53-null mutants. Statistical analysis indicated that the interaction between teratogen and genotype was highly significant (P : 0.001) for cell death on day 8 and eye defects on day 17. Conclusions: We conclude that teratogen induction of p53-dependent apoptosis in the developing embryo is positively coupled to the determination of congenital eye defects.

MISCELLANEOUS

Akita M, Murata E, Merker HJ, Kaneko K. **Morphology of capillary-like structures in a three-dimensional aorta/ collagen gel culture**. Anat Anz 1997;179(2):127-36.
The morphology of capillary-like tubes was investigated by electron microscopy (TEM and SEM) using an in vitro model of capillarogenesis (aorta/collagen type I gel). This model allowed morphological comparisons with in vivo capillaries and an evaluation of the functional maturity of the endothelium to be made. The lumina developing in vitro were demarcated by endothelial cells of varying thickness (0.1-2 microns). Pericytes were resting on the outside. The endothelial cells were characterized by contacts of varying length with tight and gap junctions and occasional indentations. The inner surface exhibited areas both with pronounced and without any endocytotic activity. In addition to a large Golgi apparatus, a varying number of cell organelles occurred depending on the thickness of the endothelium. Bundles consisting of microfilaments were often located underneath the outer cell membrane and in the vicinity of contact areas. A lamina densa was in the process of formation. The capillaries grown in vitro closely resembled those in vivo and showed a high degree of differentiation. Hence, this in vitro model allows the study of a number of functions of endothelial cells.

Altenburger R, Boedeker W, Faust M, Grimme LH. **Regulations for combined effects of pollutants: consequences from risk assessment in aquatic toxicology**. Food Chem Toxicol 1996;34(11-12):1155-7.
In the analysis of combined effects two reference concepts are currently considered as equally valid for the assessment of mixture toxicities: these are LOEWE additivity (concentration addition) and BLISS independence (response addition) (Greco et al., 1995). The aim of this study of 137 binary mixtures of pesticides and surfactants using an algal biotest was to find rational procedures for the assessment of mixture toxicities in the aquatic environment. By introducing an index on prediction quality the quantitative relationships between predicted and observed effects are evaluated for each concept. It is shown that LOEWE additivity leads to good predictions of mixture toxicities for most combinations, whereas BLISS independence tends to underestimate mixture toxicities. By this it is reaffirmed that there is a solid basis for forthcoming regulatory activities on mixtures of chemicals.

An DM, Hwang KS. [**Expert system for predicting hazardous conditions of chemical process**]. Hwahak Konghak 1996;34(6):727-34. (Kor)

Bolt HM, Mumtaz MM. **Risk assessment of mixtures and standard setting: working towards practical compromises**. Food Chem Toxicol 1996;34(11-12):1179-81.
Basic concepts of 'dose/concentration additivity' and 'response addition/independence' may be applied to evaluate chemical mixtures in human toxicology, as well as in ecotoxicology. In the case of compounds that cause the same toxicological effect by the same mechanism, 'dose addition' is a more plausible form of joint action than 'response addition'. Data on the effects of halogenated hydrocarbons on the kidney, the effects of organic solvents on the central nervous system, and the effects of organophosphates on cholinesterase, are the basis of this assumption. For such compounds, response addition will generally underestimate risk. None the less, both dose addition and response addition are 'non-interactive' forms of joint action. As such, neither additivity assumption will accurately predict risk for compounds that exhibit toxicological interactions regardless of the primary mechanism(s) of toxicity. More often, the additivity approach overestimates the risk of a combination of chemicals. From a public health perspective, such results over-protect the public; hence this approach can be used for standard setting. The introduction of a special safety factor of 10 for the standard setting for mixtures in addition to those normally used for deriving acceptable daily intakes, reference doses or minimal risk levels is not supported by data. Instead, each exposure scenario should be considered on a case-by-case basis. Furthermore, considerations of multiple endpoints, including multiple organs, multiple effects, multiple mechanisms and potential interactions between such mechanisms, are very desirable for overall toxicity and risk assessment of chemical mixtures. In conclusion, additivity could be used to estimate potential risks for a combination of chemicals: only if scientific data support independence of effects can response independence be used as an alternative for standard setting.

Campbell DB. **Extrapolation from animals to man: the integration of pharmacokinetics and pharmacodynamics**. Ann N Y Acad Sci 1996;801:116-35.
CBAC COPYRIGHT: CHEM ABS A review with many refs. This paper has focused on the difficulties of extrapolating toxicol. or pharmacol. data obtained from animals to those expected in man. For some drugs, under certain conditions, there may be no problem, but for many, this is clearly not the case. Differences in apparent activity are impossible to reconcile without normalizing the dose for differences in pharmacokinetics and metab. The increasing use of artificial intelligence and expert systems in drug investigations may provide a greater insight into why these differences may occur and allow prediction but, in the end, they must be tested in the expts. undertaken. The use of kinetic dynamic relationships in different species will certainly help in this regard and, wherever possible, should be included in exptl. design. However, one must interpret with caution the data produced by those that continue to extrapolate animal data to humans without some attempt. to discuss in detail the validity of their assumptions.

Georgopoulos PG, Walia A, Roy A, Lioy PJ. **Integrated exposure and dose modeling and analysis system. 1. Formulation and testing of microenvironmental and pharmacokinetic components**. Environ Sci Technol 1997;31(1):17-27.
BIOSIS COPYRIGHT: BIOL ABS. The conceptual and theoretical framework for a modular integrated Exposure and Dose Modeling and Analysis System (EDMAS) has been formulated, and its stepwise implementation and testing is currently in progress. This system aims to provide state-of-the-art tools for performing integrated assessments of exposure and dose for individuals and populations. The integration of modeling components with each other as well as with available environmental, exposure, and toxicological databases is being accomplished with the use of computational tools that include interactive simulation environments, Geographical Information Systems, and various data retrieval, management, statistical analysis, and visualization methods. This paper overviews the structure and modular nature of this integrated modeling system and focuses specifically on two of its components: (a) a hierarchy of physiologically based pharmacokinetic models (PBPKM), representing various levels of detail and sophistication, and (b) a family of microenvironmental models, that incorporate complex physical and chemical transformations. The deterministic implementation of these components is also presented here in two test applications: (i) a case study of benzene exposure indoors resulting from the volatilization of contaminated tap water and (ii) a case study of photochemical pollution infiltration indoors, in an office building environment.

Inoue T. [**The use of biotechnological recombinant-mice in biological safety research**]. Eisei Shikenjo Hokoku 1996;(114):1-12. (Jpn)
Number of transgenic and knock-out mice increased rapidly during the last decade. This review article describes a potential usefulness of transgenic and knock-out mice for biological safety research with respect to each toxicological category for safety evaluations, such as studies for carcinogenicity, general toxicology, genotoxicologic testing, and immuno-toxicological evaluations. In the carcinogenicity, a possible model required for a short-term study in carcinogenicity was discussed. Further, a couple of future subjects were focused specifically on the biotechnology-derived pharmaceuticals and the biotechnical recombinant-mice as a second generation, i.e. experimental mice with double or multiple gene-recombination. Those usefulnesses were also introduced briefly. Establishing the biotechnical recombinant-mice for each safety testing contributes not only to simplify and qualify the on-going evaluation system, but also to the traditional animal studies to be re-evaluated, so that the solutions may lead them to a future in vitro-alternative system much smoothly. For general references, historical reviews on the biotechnical recombination in experimental animals were also briefly introduced to elucidate a new broad area in developmental biology.

Jones DT, Morris MD, Hasan JS. **Modeling marrow damage from response data: evolution from radiation biology to benzene toxicity**. Environ Health Perspect 1996;104(Suppl 6):1293-301.
Consensus principles from radiation biology were used to describe a generic set of nonlinear, first-order differential equations for modeling toxicity-induced compensatory cell kinetics in terms of sublethal injury, repair, direct killing, killing of cells with unrepaired sublethal injury, and repopulation. This cellular model was linked to a probit model of hematopoietic mortality that describes death from infection and/or hemorrhage between 5 and 30 days. Mortality data from 27 experiments with 851 dose-response groups, in which doses were protracted by rate and/or fractionation, were used to simultaneously estimate all rate constants by maximum-likelihood methods. Data used represented 18,940 test animals: 12,827 mice, 2925 rats, 1676 sheep, 829 swine, 479 dogs, and 204 burros. Although a long-term repopulating hematopoietic stem cell is ancestral to all lineages needed to restore normal homeostasis, the dose-response data from the protracted irradiations indicate clearly that the particular lineage that is critical to hematopoietic recovery does not resemble stemlike cells with regard to radiosensitivity and repopulation rates. Instead, the weakest link in the chain of hematopoiesis was found to have an intrinsic radioresistance equal to or greater than stromal cells and to repopulate at the same rates. Model validation has been achieved by predicting the LD50 and/or fractional group mortality in 38 protracted-dose experiments (rats and mice) that were not used in fitting of model coefficients.

Klimisch HJ, Andreae M, Tillmann U. **A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data**. Regul Toxicol Pharmacol 1997;25(1):1-5.
BIOSIS COPYRIGHT: BIOL ABS. The evaluation of the quality of data and their use in hazard and risk assessment as a systematic approach is described. Definitions are proposed for reliability, relevance, and adequancy of data. Reliability is differentiated into four categories. Criteria relating to international testing standards for categorizing reliability are developed. A systematic documentation of evaluating reliability especially for use in the IUCLID database is proposed. This approach is intended to harmonize data evaluation processes worldwide. It may help the expert in subsequent assessments and should increase the clarity of evaluation.

Kobayashi K. **A comparison of one- and two-sided tests for judging significant differences in quantitative data obtained in toxicological bioassay of laboratory animals**. J Occup Health 1997;39(1):29-35.
BIOSIS COPYRIGHT: BIOL ABS. Since there are many ambiguous statements concerning the selection of one- or two-sided tests in the statistical analysis of toxicological data, I examined the rate of appearance of significant differences in the data showing a trend in either a fixed direction or a mixed sided direction compared with the control and the number of significant differences to these two tests by t and Dunnett's tests in a long-term chronic/carcinogenicity study conducted at the An-Pyo Center, in addition to referring to the most widely used statistical analyses by mean of the one-or two-sided test in the literature. The results were as follows; (1) Almost all quantitative data (578 out of 700 cases) showed a fixed trend with statistically significant differences ($p < 0.05$) compared with the control value. (2) The number of significant differences obtained with the one-sided test was

greater than with the two-sided test in either analysis of the T or Dunnett's test; that is, the percentages of the significant differences in the two-sided test were 85 and 86% of those in one-sided test by means of T and Dunnett's test, respectively. (3) The frequency of use of the one-sided tests was very low in both Japanese and international publications. Consequently, the one-sided test may be recommended for statistical analyses of toxicological bioassay data that generally show a fixed trend as compared with the control values, since more rigid evaluation of the data of the chemical effects on the living body and the environment is necessary.

Kurosawa S, Tawara-Kondo E, Kamo N. **Detection of mutagenic polycyclic compounds using a piezoelectric quartz crystal coated with plasma-polymerized phthalocyanine derivatives**. Anal Chim Acta 1997;337(1):1-3. BIOSIS COPYRIGHT: BIOL ABS. Many polycyclic compounds such as the products of amino acid pyrolysis are mutagens. We devised a system for selective detection of these compounds in solution, based on a piezoelectric quartz crystal coated with plasma-polymerized Fe phthalocyanine or Fe tetraphenylporphine films.

Nalecz-Jawecki G, Rudz B, Sawicki J. **Evaluation of toxicity of medical devices using Spirotox and Microtox tests. I. Toxicity of selected toxicants in various diluents**. J Biomed Mater Res 1997;35(1):101-5.

Schoen ED. **Statistical designs in combination toxicology: a matter of choice**. Food Chem Toxicol 1996;34(11-12): 1059-65.