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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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GENERAL

Barratt MD, Chamberlain M. **Integration of QSAR and in vitro toxicology**. In Vitro Methods Pharm Res 1997;15-31.
CBAC COPYRIGHT: CHEM ABS A review, with 48 refs., discussing ways in which QSAR studies and in vitro toxicol. methods can complement each other in assessing toxicity as alternatives to live-animal expts. The value of the mechanistic approach to QSAR and in vitro methods is examd.; different types of predictive models are discussed, together with ways in which they might be integrated. Criteria for the selection of sets of chems. for test development, optimization, and validation are suggested; proposals are made for ways in which QSAR studies might be used to support the use of data from in vivo tests which do not meet current requirements of acceptability. Ways of assessing the mechanistic competence of in vitro assays and of improving their design are discussed.

Dolfini E, Lavazza M, Meloni M. **Cytotoxicity and antiaging activity of some cosmetic compounds: a pool of in vitro tests on two human immortalized cell lines, NCTC 2544 and WI-38**. In Vitro Toxicol 1997;10(1):55-61.
CBAC COPYRIGHT: CHEM ABS This research presents an in vitro model in a pool of tests to study some biol. activities on 2 human immortalized cell lines: keratinocytes (NCTC 2544) and fibroblasts (WI 38). The tests were set up to analyze the cytotoxicity, efficacy, cytoskeleton protein alterations, and the prolonging of in vitro cell life of hyaluronic acid, eramides, and 2 patented active anti-free radicals cosmetic substances: Plusina and its lipophilic deriv. Plusina 2000. The nontoxic dose of each compd. was chosen to study the biol. alterations of some growth parameters. In the serum-free assay, calf serum was substituted in culture medium for the nontoxic dose of each compds. The highest activity on the cellular growth was shown by the ceramides (2 cellular doublings more compared to the untreated controls). The studies on the cytoskeleton protein structure showed degrdn. and partial depolymn. of cytokeratin 18 on NCTC 2544 cell line after treatment with Plusina and Plusina 2000 (10 mug/mL). The aging test on human fibroblasts WI 38 showed that only Plusina 2000 induced long extension of cellular life span of 70 days compared to untreated controls. Those tests could be of interest for a preliminary in vitro study of toxicol., efficacy, and prolonging of in vitro cell life of a large amt. of raw materials and cosmetic active substances as a possible alternative to animal testing.

Doucette GJ, Logan MM, Ramsdell JS, Van Dolah FM. **Development and preliminary validation of a microtiter plate-based receptor binding assay for paralytic shellfish poisoning toxins.** Toxicon 1997;35(5):625-36.
More than 20 countries have either established or proposed regulatory limits for one or more of the paralytic shellfish poisoning (PSP) toxins as they occur in seafood products. PSP toxin levels are generally estimated using the standard AOAC mouse bioassay, yet because of various limitations of this method [e.g. high variability (+/-20%), low sensitivity, limited sample throughput and use of live

animals], there remains a need for alternative testing protocols. A sensitive and selective, high capacity assay was developed for the PSP toxins which exploits the highly specific interaction of these toxins with their biological receptor (i.e. voltage-dependent sodium channel) and is thus based on functional activity. This receptor binding assay provides a radioactive endpoint, and is performed in a microtiter filter plate format with results determined by standard liquid scintillation counting within 24 hr. The Ki for the assay is 3.66 +/- 0.86 nM saxitoxin, with a limit of detection of c. 5 ng saxitoxin/ml in a sample extract. Good quantitative agreement of the assay with both mouse bioassay and high-performance liquid chromatographic analysis of crude extracts of contaminated shellfish, as well as PSP toxin-producing algae, was observed. Our findings indicate that the receptor binding assay has a strong predictive value for toxicity determined by mouse bioassay, and that this approach warrants consideration as a rapid, reliable and cost-effective alternative to live animal testing for detection and estimation of PSP-related toxicity in seafood and toxic algae.

Kristen U. **Use of higher plants as screens for toxicity assessment.** Toxicol In Vitro 1997;11(1-2):181-91.
BIOSIS COPYRIGHT: BIOL ABS. This review deals with the use of entire plants, seedlings, cell suspension cultures and pollen tubes for the estimation of potential toxicity in the environment, and for risk assessment of chemicals and formulations of human relevance. It is shown that the roots of onions and various crop seedlings, as well as in vitro growing pollen tubes of some mono- and dicotyledonous plants, are most frequently used to obtain toxicity data by determination of root and tube growth inhibition. Both roots and pollen tubes are chloroplast free, non-photosynthetic systems and, therefore, with regard to their cytotoxic reactions are closer to vertebrate tissues and cells than are chloroplast-containing plant organs. Root tips and anthers of flower buds are shown to be applicable to genotoxicity screening by microscopic analysis of mitotic or meiotic aberrations during cell division or microspore development, respectively. The processes of mitosis and meiosis are similar in plants and animals. Therefore, meristematic and sporogenic tissues of plants generally show patterns of cytotoxic response similar to those of embryogenic and spermatogenic tissues of vertebrates. The suitability of root tips, cell suspensions and pollen tubes for the investigation of mechanisms of toxic action and for the analysis of structure-activity relationships is also demonstrated. Two plant-based assays, the Allium test and the pollen tube growth test, both currently being evaluated alongside with established mammalian in vivo and in vitro protocols, are emphasized with regard to their potential use as alternatives to animal in vivo toxicity tests. For both assays, preliminary results indicate that the tips of growing roots and the rapidly elongating pollen tubes of certain higher plant species are as reliable as mammalian cell lines for detecting basal cytotoxicity. It is suggested that seeds and pollen grains, in particular, provide easily storable and convenient systems for inexpensive, relatively simple but precise toxicological assays.

Potera C. **Tracking toxicants.** Environ Health Perspect 1997;105(1):48-50.

Rasooly L, Rose NR, Shah DB, Rasooly A. **In vitro assay of Staphylococcus aureus enterotoxin A activity in food.** Appl Environ Microbiol 1997;63(6):2361-5.
Staphylococcus aureus enterotoxin A (SEA) is a leading cause of food poisoning. The current test for functional activity of SEA requires monkeys or kittens. The major drawbacks of animal assays are lack of quantitation, poor reproducibility, low sensitivity, and high cost. In this report we describe and

evaluate an alternative assay using T-cell proliferation to measure SEA activity in food. Human and rat lymphocytes proliferate in response to concentrations of SEA as low as 1 pg/ml, well below the pathogenic dose of 100 ng. This proliferation assay is highly sensitive, quantitative, and simple. Nonradioactive assays of T-cell proliferation were also suitable for detecting and measuring SEA, although with a 10-fold lower sensitivity. To evaluate the utility of this assay for food testing, four different food samples were mixed with SEA. In each sample, SEA was detected at a concentration of 1 ng/ml. Heat-inactivated SEA produced no detectable proliferation. These results demonstrate that an in vitro cell proliferation assay is an advantageous alternative to existing animal assays for measuring SEA activity in food.

Scott HS, Chen H, Rossier C, Lalioti MD, Antonarakis SE. **Isolation of a human gene (HES1) with homology to an Escherichia coli and a zebrafish protein that maps to chromosome 21q22.3**. Hum Genet 1997;99(5):616-23.
CBAC COPYRIGHT: CHEM ABS Exon trapping was performed with chromosome 21 cosmids to identify those that may be involved in the pathogenesis of Down syndrome, or several of the genetic diseases that map to chromosome 21. BLASTX anal. revealed two exons with significant homol. to a zebrafish protein (ES1) and an Escherichia coli protein (sigma cross-reacting protein 27A), both of unknown function. The exons also showed identity with several expressed sequence tags (ESTs). Sequences from all ESTs derived from this gene and reverse transcription-polymerase chain reaction (RT-PCR) anal. were used to det. the full cDNA sequence, which corresponded to an mRNA of 1.7 kb with an open reading frame of 268 amino acids. The mRNA from this gene, termed HES1, is ubiquitously expressed, but strongly so in heart and skeletal muscle. Potential mitochondrial targeting signals were found in both the human and zebrafish proteins, consistent with the expression levels in muscle tissues. The strong homol. between the E. coli, zebrafish and HES1 proteins suggests an important biol. role. Hybridization of RT-PCR products to a cosmid contig in chromosome 21q22.3, mapped HES1 just proximal to D21S25, a crit. mapping region for several genetic diseases. Given the mapping position, this gene is a candidate for involvement in these disorders, including autoimmune polyglandular disease type I and the autosomal nonsyndromic deafness loci, DFNB8 and DFNB10. In addn., the initial method of EST identification for gene isolation presented here is valid for many genes and can be used to obtain initial sequence contigs without cloning or library screening.

CARCINOGENESIS

Ashby J. **Cell transformation assays as predictors of carcinogenic potential [letter]**. Toxicol Pathol 1997;25(3):334-5.

Ashby J. **Identifying potential human carcinogens--the role of genetically altered rodents.** Toxicol Pathol 1997;25(2):241-3.

Balenko NV, Chernichenko IA, Yanysheva NY, Litvichenko ON, Sovertkova LS, Babii VF. **[The role of nitrogen dioxide in endogenous synthesis of carcinogenic nitrosamines]**. Eksp Onkolog 1997;19 (1):20-5. (Rus)

BIOSIS COPYRIGHT: BIOL ABS. Carcinogenic N-nitrosamines (NA) have been proved to be formed endogenously in white rats exposed to NO2 by inhalation. The incidence of tumours, localization, malignancy, multiplicity, and detection time were found to be dependent on NO2 concentration. A number of facts including high occurrence of liver tumours, detection of N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) in liver, lung, and kidney of rats during administration of NO2 and other NA precursors demonstrated their danger with relation to potential NA endogenous synthesis and carcinogenic effect. At the same time, combined exposure to NO2 plus AP at the level of maximal permissible concentration (MPC) did not influence carcinogenesis in rat.

Chou MW, Chen W, Mikhailova MV, Nichols J, Weis C, Jackson CD, Hart RW, Chung KT. **Dietary restriction modulated carcinogen-DNA adduct formation and the carcinogen-induced DNA strand breaks.** Toxicol Lett 1997; 92(1):21-30.
Dietary restriction (DR) alters the activities of hepatic drug metabolizing enzymes and modulates the formation of carcinogen-DNA adducts in carcinogen treated animals. Our previous results showed that a 40% restriction of diet (60% of ad libitum (AL) food consumption) reduced the hepatic metabolic activation of aflatoxin B1 (AFB1) but increased the activation of benzo[a]-pyrene (BaP) in both rats and mice. In this study, the focus was directed toward the levels of carcinogen-DNA adducts formation and the carcinogen-induced DNA strand breaks in mouse kidney and liver DNA. DR significantly inhibited both AFB1-DNA adduct formation and AFB1-induced DNA strand breaks in kidney DNA of mice that received a single dose of [3H]AFB1 (5 mg/kg). The levels of AFB1-DNA adduct formation in mouse kidney DNA correlated well with increased AFB1-induced DNA strand breaks. The correlation between the levels of AFB1-DNA-adducts formed and DNA strand breaks in kidney DNA of DR-mice was less linear than between its AL-counterpart suggesting that other factors, such as different rates of DNA repair, may be involved. In addition, DR enhanced hepatic BaP- and 6-nitrochrysene (6-NC)-DNA adduct formation in the mice treated with BaP and 6-NC, respectively. The formation of the specific BaP-adduct, 10-(N2-deoxyguanosinyl)-7,8,9-trihydroxy-7,8,9,10-tetrahydro-BaP (N2-dG-BaP), in mouse liver was proportional to the dose, and was compatible to the BaP-induced DNA strand breaks affected by DR. The enhancement of the total 6-NC-DNA adduct formation in DR-mouse was also in correlation with the increased 6-NC-induced DNA strand breaks. The activity of mouse liver microsomal nitro-reductase increased by 2-fold in response to DR indicating that the nitroreduction may contribute to the increase of themetabolic activation of 6-NC. Our present results indicate that the effect of DR on the carcinogen activation is dependent upon the DR-modulated carcinogen metabolizing enzyme activities.

Coker DT, King AG, Mumford DL, Nessel CS. **Carcinogenic assessment of petroleum products by nuclear magnetic resonance.** Anal Commun 1997;34(5):137-40.
BIOSIS COPYRIGHT: BIOL ABS. A method is proposed for improving the assessment of carcinogenicity of petroleum materials by non-animal test means. The proposed method is an extension of the current EU regulatory test method IP 346 which measures a proportion of the polycyclic aromatic compounds present, but does not discriminate between the carcinogenic and the non-carcinogenic species, sometimes resulting in false positive assessments. The proposed method involves the use of high-field proton NMR spectrometry on the IP 346 solvent extract to measure the level of 'bay region hydrogens' within polycyclic aromatic molecules. These are structures within the molecule, considered

to be directly associated with their carcinogenic potential. Tests on a wide range of petroleum materials using this proton NMR method show that the bay region hydrogen measurement gives greater discrimination between carcinogenic and non-carcinogenic materials than IP 346. The NMR method has not so far given any of the false positives which occur with IP 346 for oils which are not found to be carcinogenic by bioassay. It may also be possible to use the bay region hydrogen measurement as a marker for carcinogenicity in a range of petroleum products which are outside the scope of IP 346.

Contrera JF, Jacobs AC, DeGeorge JJ. **Carcinogenicity testing and the evaluation of regulatory requirements for pharmaceuticals.** Regul Toxicol Pharmacol 1997;25(2):130-45.
The results of rat and mouse carcinogenicity studies for 282 human pharmaceuticals in the FDA database were analyzed and compared as part of an International Conference on Harmonization (ICH) evaluation of rodent carcinogenicity studies and their utility for carcinogenicity testing. A majority of the carcinogenicity studies in the FDA database were carried out in Sprague-Dawley-derived rats and Swiss-Webster-derived CD-1 mice in contrast to Fisher 344 rats and B6C3F1 mice employed in National Toxicology Program (NTP) studies. Despite the differences in rodent strains, the relative proportion of compounds with positive findings (44.3%) and the degree of overall concordance between rats and mice (74.1%) in the FDA database were similar to the NTP rodent carcinogenicity database. Carcinogenicity studies in two rodent species are necessary primarily to identify trans-species tumorigens, which are considered to pose a relatively greater potential risk to humans than single species positive compounds. Two-year carcinogenicity studies in both rats and mice may not be the only means of identifying trans-species tumorigens. Sufficient experience is now available for some alternative in vivo carcinogenicity models to support their application as complementary studies in combination with a single 2-year carcinogenicity study to identify trans-species tumorigens. Our analysis of the rodent carcinogenicity studies supports such an approach for assessing carcinogenic potential without compromising the public health.

Cunningham ML, Matthews HB. **Cell proliferation as a determining factor for the carcinogenicity of chemicals: studies with mutagenic carcinogens and mutagenic noncarcinogens**. Toxicol Lett 1995;82-83:9-14.
Efforts to develop a cheaper, faster method for determining the carcinogenic potential of chemicals have been undertaken using comparisons of mutagenic structural analogs of carcinogenic and noncarcinogenic chemicals. Results of short term in-vitro tests for mutagenicity were compared with data from rodent in-vivo bioassays. Carcinogenicity was found to be associated with the chemical isomer's ability to cause cell proliferation in target organs. The mutagenic carcinogen analogs 2,4-diaminotoluene (95807) (2,4-DAT), 2-nitropropane (79469), and tris(2,3-dibromopropyl)phosphate (126727) were all found to produce cell proliferation, while mutagenic noncarcinogens such as 2,6-diaminotoluene (823405) (2,6-DAT), 1-nitropropane (108032), dimethoate (60515), dioxathion (78342), and dichlorvos (62737) did not. Studies using transgenic mice have demonstrated that the carcinogenic isomer 2,4-DAT produces an increase in mutation frequency but that the noncarcinogenic isomer 2,6-DAT did not. These results suggest that cell proliferation may be a necessary factor in the expression of chemically induced mutagenicity.

Dickson JR, Brinkman DW, Blackburn GR. **Evaluation of the dermal carcinogenic potential of re-**

refined base stocks using the modified Ames assay, PAC analysis and the 32P-postlabeling assay for DNA adduct induction. J Appl Toxicol 1997;17(2):113-7.
The standard method for assessing the carcinogenicity of lubricating oil base stocks is the mouse skin-painting bioassay. This assay has the advantage of directly measuring the endpoint of interest, dermal carcinogenicity, but has the drawback of being time-consuming and expensive. For this reason, a variety of biological and chemical assays have been developed as predictive alternatives to the in vivo assay. This publication describes the application of three such methods to the assessment of carcinogenic potential of hydrotreated, re-refined oils: the modified Ames test, the analytical determination of 3-7-ring polycyclic aromatic compound content and the 32P-postlabeling assay for DNA adduct induction.

Dybing E, Sanner T, Roelfzema H, Kroese D, Tennant RW. **T25: a simplified carcinogenic potency index: description of the system and study of correlations between carcinogenic potency and species/site specificity and mutagenicity.** Pharmacol Toxicol 1997;80(6):272-9.
A simplified carcinogenic potency index, the T25, is proposed as a practical method for the inclusion of potency considerations in carcinogen classification systems. The T25 is the chronic daily dose in mg per kg bodyweight which will give 25% of the animals tumours at a specific tissue site, after correction for spontaneous incidence, within the standard life span of that species. Calculated T25 values of a set of 113 US National Cancer Institute/National Toxicology Program (NC/NTP) carcinogens showed excellent correlation (correlation coefficient 0.96, $P < 0.0001$) with the carcinogenic potency index TD50 of Peto et al. (1984). The mean of T25 values for 51 transspecies, multiple common site NCI/NTP carcinogens were 10-fold lower than those for 62 NCI/NTP single species, single site carcinogens. For these 113 carcinogens, the mean T25 values were approximately 3-fold lower for agents that were also mutagenic in Salmonella compared to the non-mutagenic agents.

Eder E, Budiawan, Schuler D. **Crotonaldehyde: a carcinogenic and mutagenic air, water and food pollutant.** Cent Eur J Public Health 1996;4(Suppl):21-2.
Crotonaldehyde is mutagenic and carcinogenic and it is ubiquitous in our environment. The data base does, however, not allow an assessment of the carcinogenic risk. We have developed a sensitive 32P-postlabelling technique which allows the detection of specific DNA-adducts in animal tissues as markers for initiation of cancer cells. Adducts were found in several organs of F 344 rats after gavage and persisted to a certain extent. The determination of adduct levels in animal tissues after different exposure or even in human tissues can therefore be considered as an effect monitoring and would certainly improve the risk assessment.

Fetterman BA, Kim BS, Margolin BH, Schildcrout JS, Smith MG, Wagner SM, Zeiger E. **Predicting rodent carcinogenicity from mutagenic potency measured in the Ames Salmonella assay.** Environ Mol Mutagen 1997;29(3):312-22.
BIOSIS COPYRIGHT: BIOL ABS. Many in vitro tests have been developed to identify chemicals that can damage cellular DNA or cause mutations, and secondarily to identify potential carcinogens. The test receiving by far the most use and attention has been the Salmonella (SAL) mutagenesis test developed by Ames and colleagues ((1973): Proc Natl Acad Sci USA 70:2281-2285; (1975): Mutat Res 31:3A7-364), because of its initial promise of high qualitative (YES/NO) predictivity for cancer in rodents and, by extension, in humans. In addition to the initial reports of high qualitative predictivity, there was also

an early report by Meselson and Russell (in Hiatt HH et al. (1977): Origins of Human Cancer, Book C: Human Risk Assessment, pp 1473-1481) of a quantitative relationship between mutagenic potency measured in SAL and carcinogenic potency measured in rodents, for a small number of chemicals. However, other reports using larger numbers of chemicals have found only very weak correlations. The primary purpose of this study was to determine whether mutagenic potency, as measured in a number of different ways, could be used to improve predictivity of carcinogenicity, either qualitatively or quantitatively. To this end, eight measures of SAL mutagenic potency were used. This study firmly establishes that the predictive relationship between mutagenic potency in SAL and rodent carcinogenicity is, at best, weak. When predicting qualitative carcinogenicity, only qualitative mutagenicity is useful; none of the quantitative measures of potency considered improves the carcinogenicity prediction. In fact, when qualitative mutagenicity is forced out of the model, the quantitative measures are still not predictive of carcinogenicity. When predicting quantitative carcinogenicity, several possible methods were considered for summarizing potency over all experiments; however, in all cases, the relationship between mutagenic potency predictors and quantitative carcinogenicity is very weak.

Fujiwara K, Koike H, Ohishi Y, Shirafuji H, Kohno I, Modest EJ, Kataoka S. **Cytokinetic and morphologic differences in ovarian cancer cells treated with ET-18-OCH3 and the DNA-interacting agent, etoposide.** Anticancer Res 1997;17(3c):2159-67.
New antineoplastic agents with different cytotoxic mechanisms are of interest for their ability to overcome resistance to conventional DNA-interacting agents. Ether lipids are known to be active against ovarian carcinoma both in vitro and in vivo, and the cell membrane is believed to be the target of their antitumor activity. In this study we have investigated the different cytokinetic and morphologic responses of human ovarian carcinoma cells (BG-1) to one of the ether lipids (ET-18-OCH3) and to etoposide. Etoposide induced a significantly greater G2/M block. However, the proportion of the cycling cell fraction decreased significantly in cells treated by ET-18-OCH3 and induction of the hypodiploid traction was strongly correlated with reduction of the cycling cell fraction. On the other hand, the hyperdiploid fraction was found to correlate with reduction of the cycling cell fraction in etoposide treated cells. Despite the significant appearance of the hypodiploid fraction, apoptosis was not observed by DNA-gel assay. Microscopic study showed that the hyperdiploid fraction represented cells with multiple nuclei. These observations support the unique lethal effect of ET-18-OCH3 on ovarian carcinoma cells, distinguishing it from the action of a typical DNA-interacting agent. The membrane-targeted ether lipids deserve consideration for the future chemotherapy of ovarian carcinoma, perhaps in combination with the appropriate DNA-interacting agent. New antineoplastic agents with different cytotoxic mechanisms are of interest not only for their unique inhibitory properties but also for their potential of overcoming resistance to conventional DNA-interacting agents. Ether lipids are known to be active against ovarian carcinoma both in vitro (1, 2, 3) and in vivo (4, 5), and the cell membrane is believed to be the target of their antitumor activity. Etoposide, a DNA-interacting agent, is also active against human ovarian cancer cells in vitro (6) or in clinical trials either as a single agent (7) or in combination with cisplatin (8). We have reported that a cytotoxic dose of one of the ether lipids ET-18-OCH3, induces a G2/M block in BG-1 human ovarian cancer cells, and also a hypodiploid fraction as shown on DNA analysis by flow cytometry (FCM) (9). The G2/M block was also observed in BG-1 cells following etoposide treatment (6). In the present study, we have investigated the differences in the

cytokinetic and morphologic responses of BG-1 cells to ET-18-OCH3 and to etoposide.

Funk M, Ponten I, Seidel A, Jernstrom B. **Critical parameters for adduct formation of the carcinogen (+)-anti-benzo[a]pyrene-7,8-dihydrodiol 9,10-epoxide with oligonucleotides.** Bioconjug Chem1997;8(3):310-7.
Various parameters relevant for the formation of dG adducts produced in the reaction of individual benzo [a]pyrene diol epoxide (BPDE) stereoisomers with oligonucleotides have been studied. Reaction time, temperature, pH, molar ratio of diol epoxide and oligonucleotide, base sequence, and buffer system were shown to affect the amount of (+)-anti-BPDE dG adducts formed. Optimum experimental conditions for dG adduct formation were different depending on the base sequence context of the oligonucleotide employed [5'-d(CCTATAGATATCC) or 5'-d(CCTATTGCTATCC)]. In general, low temperature to allow a longer reaction time, slightly alkaline Tris-HCl (pH 7.5-8.0) or alkaline phosphate buffer (pH 11), low concentration of organic solvent, and a molar excess of (+)-anti-BPDE promote dG adduct formation with an oligonucleotide. Low incubation temperature and Tris-HCl buffer also favor dG adduct formation of (-)-anti-BPDE and both enantiomers of syn-BPDE to both 5'-d (CCTATAGATATCC) and 5'-d(CCTATTGCTATCC).

Huang G, Xiao X, Huang Y, Huang R. **[Carcinogenic mechanisms of multiple genes in cervical carcinoma]**. Hua Hsi I Ko Ta Hsueh Hsueh Pao 1996;27(1):5-9. (Chi)
The alterations of multiple genes and their carcinogenic mechanism in cervical carcinoma were studies by molecular hybridisation, PCR and PCR-ASO techniques. The G-T point mutation in the 12th coden of Ha-ras was detected in cervical carcinomas with mutation frequency of 18.2% (8/44), and the amplification rate of Ha-ras gene was 45% (9/20). The c-erb B2 was amplified 3-30 fold with an amplification rate of 73.3% (11/15) in cervical carcinomas and 5 cancerous samples showed gene rearrangement. The elevated copies of c-myc gene with amplification rate of 91.7% (11/12) were observed in cervical carcinomas. The study of HPV16 viral gene showed that the existence of HPV16 DNA sequence was positively associated with c-myc gene amplification in cervical cancerous samples. The p53 and Rb tumor suppressor genes absence of deletion were observed in the 12 specimens of cervical carcinoma investigated. As mentioned above, the study on alteration and carcinogenic mechanism of multiple genes indicated that 3 oncogenes and HPV16 viral gene were activated or integrated throygh different mechanisms and they played roles in co-carcinogenesis. The integration of HPV16 gene might promote the c-myc gene at the early stage in carcinogenesis of cervical carcinoma, while the alteration of Ha-ras and c-erb B2 gene might be middle-late event. As for the roles of the p53 and Rb tumor suppresor gene in cervical carcinogenesis need further researches.

Liehr JG. **Dual role of oestrogens as hormones and pro-carcinogens: tumour initiation by metabolic activation of oestrogens.** Eur J Cancer Prev 1997;6(1):3-10.
Epidemiological evidence increasingly points to exogenous or endogenous oestrogens as a risk factor for breast cancer. However, it is unlikely that induction of oestrogen-dependent tumour growth is the sole contribution of oestrogens to tumour development in the mammary gland, because oestrogen receptors are barely detectable in normal mammary epithelial cells. In this review, I examine a mechanism for mammary carcinogenesis, which emphasizes tumour initiation by metabolic activation of oestrogens in combination with cell transformation and growth stimulation by oestrogen receptor-mediated processes.

Catecholestrogen metabolites are capable of metabolic redox cycling between quinone and hydroquinone forms, a mechanism of free radical generation. Several types of direct and indirect free radical-mediated DNA damage are induced by oestrogens in vitro and in vivo, such as DNA single strand breaks, 8-hydroxylation of guanine bases, and DNA adduct formation by malondialdehyde, a decomposition product of free radical-induced lipid peroxides. The substrate for redox cycling and free radical generation may be 4-hydroxoestradiol, because this metabolite is formed from oestradiol by a specific oestrogen 4-hydroxylase detected in several human organs including mammary tissue. It has also been formed in organs of rodents where oestrogens induce tumours, with the exception of the liver. 4-Hydroxyoestradiol is a potent, long-acting oestrogen and may complete the carcinogenic process by stimulating receptor-mediated proliferation. An understanding of a possible mechanism of mammary carcinogenesis as a result of oestrogen-mediated initiation means that several prevention strategies, based on inhibiting metabolic activation of oestrogens or free radical action, can be developed.

Mehta RG, Hawthorne ME, Steele VE. **Induction and prevention of carcinogen-induced precancerous lesions in mouse mammary gland organ culture.** Methods Cell Sci 1997;19(1):19-24.
BIOSIS COPYRIGHT: BIOL ABS. Mouse mammary glands respond to growth promoting hormones in organ culture. In the presence of insulin, prolactin, aldostrone, and hydrocortisone, the glands exhibit extensive proliferation within 10 days of culture mimicking the mammary alveolar structures observed during pregnancy. However withdrawal of prolactin and steroids from the medium for an additional 14 days results in the disintegration of the alveolar structures resembling the mammary morphology observed during the involution stage. During the growth promoting phase if the glands are exposed to 7, 12, dimethyl-benz(a)anthracene (DMBA) for 24 hours and cultured through the entire 24 days of culture period, they develop precancerous lesions. This model is highly reproducible and extensively utilized to evaluate efficacy of potential chemopreventive agents against carcinogen-induced mammary lesions.

Nakao T, Yoshimura T, Kurita C, Watanabe S, Horiba M, et al. **[Chemosensitivity testing of lung cancer cells using the MTT assay]**. Jpn J Hosp Pharm 1996;22(4):380-9. (Jpn)
IPA COPYRIGHT: ASHP The adaption of a simple colorimetric test, the MTT assay, for chemosensitivity testing of a human lung adenocarcinoma cell line against a variety of antineoplastic drugs is described.

Nomura T, Nakajima S, Kawabata K, Yamashita F, Takakura Y, Hashida M. **Intratumoral pharmacokinetics and in vivo gene expression of naked plasmid DNA and its cationic liposome complexes after direct gene transfer**. Cancer Res 1997;57(13):2681-6.

Oikawa T, Sasaki M, Inose M, Shimamura M, Kuboki H, Hirano S, Kumagai H, Ishizuka M, Takeuchi T. **Effects of cytogenin, a novel microbial product, on embryonic and tumor cell-induced angiogenic responses in vivo**. Anticancer Res 1997;17(3c):1881-6.

Ojala WH, Iyer RA, Hanna PE, Gleason WB. **Heterocyclic N-acetoxyarylamines, models for the putative ultimate carcinogens of aromatic amines: 2-acetoxyamino-5-phenylpyridine and 2-acetoxyaminopyridine.** Acta Crystallogr C 1997;53(Pt 5): 634-7.

The structures of O-acetyl-N-(5-phenyl-2-pyridyl)-hydroxylamine, C13H12N2O2, (I), and O-acetyl-N-(2-pyridyl)hydroxylamine, C7H8N2O2. (II), have been determined in order to confirm earlier structure assignments based on spectroscopic information. Compound (I) is the probable mutagenic metabolite of the phenylalanine pyrolysis product 2-amino-5-phenyl-pyridine. The crystal structures of (I) and (II) are the first reported for heterocyclic N-acetoxyarylamines, the corresponding homocyclic arylamine derivatives being extremely unstable. In the solid state, both (I) and (II) exist as hydrogen-bonded dimers, with the arylamine N atom acting as donor and the pyridine N atom of a neighboring inversion-related molecule as acceptor; the distance between donor and acceptor N atoms is 3.007(2) in (I) and 2.956(2) A in (II). This orientation of the N-H bond results in the rotation of the acetoxy group out of the plane of the pyridine ring by 22.5(2) in (I) and 27.4(2) degrees in (II).

Okada G, Ryoyama K, Nomura T, Momoi T, Tsuchiya H, Kameyama T, Yamaguchi K. **Carcinogen-induced de novo methylation in c-myc exon I.** Jpn J Med Sci Biol 1996;49(5-6):209-18.
During the response of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), methylation occurred at the Hpa II site of c-myc exon I, which is located downstream of the P1 initiation site, as evidenced by the assays of Hpa II-PCR. The Hpa II spite of the 5' flanking region did not undergo methylation. UV-irradiation also led to methylation in exon I. The extent of methylation increased depending on the dose of MNNG and UV. The results suggested that methylation takes place in transcriptionally active c-myc responsible for carcinogens and is caused by mechanisms different from that of alkylation in a specific CpG site. Possible contribution of methylation to less repair found in c-myc is discussed.

Page NP, Singh DV, Farland W, Goodman JI, Conolly RB, Andersen ME, Clewell HJ, Frederick CB, Yamasaki H, Lucier G. **Implementation of EPA revised cancer assessment guidelines: incorporation of mechanistic and pharmacokinetic data.** Fundam Appl Toxicol 1997;37(1):16-36.
BIOSIS COPYRIGHT: BIOL ABS. A workshop entitled Implementation of EPA Revised Cancer Assessment Guidelines: Incorporation of Mechanistic and Pharmacokinetic Data was held in Anaheim, California, in 1996 at the 35th Annual Meeting of the Society of Toxicology (SOT). This workshop was jointly sponsored by the Carcinogenesis, Risk Assessment, and Veterinary Specialty Sections of the SOT. The thrust of the workshop was to discuss the scientific basis for the revisions to the EPA Guidelines for cancer assessment and EPA's plans for their implementation. This is the first revision to the original EPA guidelines which have been in use by EPA since 1986. The principal revisions are intended to provide a framework for an increased ability to incorporate biological data into the risk assessment process. Two cases were presented, for chloroform and trichloroethylene, that demonstrated the use of the revised guidelines for specific cancer risk assessments. Using these new guidelines, nonlinear margin of exposure analyses were proposed for these chemicals instead of the linearized multistage model previously used by the EPA as the default method. The workshop participants generally applauded the planned revisions to the EPA guidelines. For the most part, they considered that the revised guidelines represented a positive step which should allow for and encourage the use of biological information in the conduct of cancer risk assessments. Several participants cautioned however that the major problem with cancer risk assessments would continue to be the inadequacy of available data on which to conduct more scientific risk assessments.

Patlak M. **Fingering carcinogens with genetic evidence**. Environ Sci Technol 1997;31(4):190-2.

BIOSIS COPYRIGHT: BIOL ABS. RRM JOURNAL ARTICLE HUMAN CARCINOGENS GENETICS DNA TUMOR BIOLOGY MUTATIONS.

Pliss MB, Yatskovskaya NY, Gulich MP, Solomko GI, Solov'eva VF. **[Understanding possible carcinogenic action of a protein concentrate from Saccharomyces yeast grown in molasses]**. Likars'Ka Sprava 1996;(10-12):107-11. (Rus)
BIOSIS COPYRIGHT: BIOL ABS. A testing was done in a chronic experiment on 300 rats and 360 mice of both sexes for carcinogenic potential of a new protein product from Saccharomyces yeast grown in melasse. The production procedures and techniques of the above product have been worked out at the Ukrainian Research Institute of Spiritus and Biotechnology of Food Stuffs of Gospishcheprom'a (State Food Industry) of Ukraine. The studies made showed the new protein product has no carcinogenic effect.

Pool-Zobel BL, Leucht U. **Induction of DNA damage by risk factors of colon cancer in human colon cells derived from biopsies**. Mutat Res 1997;375(2):105-15.
CBAC COPYRIGHT: CHEM ABS To increase the understanding of the factors responsible for causing human colon cancer, a technique was developed to detect genotoxic effects of chems. in human colon cells. Risk factors suspected to be assocd. with the etiol. of human colon cancer were subsequently investigated: the method is based on the measurement of DNA damage in primary cells freshly isolated from human colon biopsies with the single cell microgel electrophoresis technique ('Comet Assay'). 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 2-amino-3-methyl-3H-imidazo[4,5f]quinoline (IQ), N-methyl-N-nitro-N-nitrosoguanidine (MNNG), dinitrosocaffeidine (DNC) lithocholic acid (LCA), hydrogen peroxide (H2O2) and benzo[a]pyrene (B[a]P) were investigated for their genotoxic and cytotoxic effects following 30 min incubation with colon cells of human, and for comparative purposes also of the rat colon. The nitrosamides (MNNG, DNC) were very genotoxic in human colon cells. MNNG was more genotoxic in human than in rat colon cells. In contrast, the rat colon carcinogens PhIP and IQ were not genotoxic in human colon cells. PhIP did induce DNA damage in rat colon cells, which correlates to its capacity of inducing tumors in this animal tissue. LCA was toxic (rat>human) and concomitantly caused DNA damage in higher concns. The widespread contaminant B[a]P was not genotoxic in colon cells of either species using this system. H2O2 was found to be a potent genotoxic agent to both rat and human colon cells (human>rat). In summary, those compds. chosen as representatives of endogenously formed risk factors (MNNG, H2O2, LCA) have a higher toxic and/or genotoxic potency in human colon tissue than in rat colon. They are also more effective in this system than the contaminants tested so far (B[a]P, PhIP, IQ). The newly developed technique is rapid and yields relevant results. It is a novel and useful approach to assess different chem. compds. for genotoxic activities in tumor target tissues of the human.

Ragg SJ, Woods GM, Egan PJ, Dandie GW, Muller HK. **Failure of carcinogen-altered dendritic cells to initiate T cell proliferation is associated with reduced IL-1 beta secretion.** Cell Immunol 1997;178 (1):17-23.
The activation of T cells through presentation of antigen by dendritic cells (DC) relies on many factors, including the correct balance of cytokines in the immediate microenvironment. Antigen presentation by DC migrating from carcinogen-treated skin is impaired as evidenced by the failure of antigen-pulsed DC

to initiate specific T cell proliferation. To elucidate mechanism(s) of DC dysfunction, DC migrating from carcinogen-treated skin were collected, pulsed with OVA, and cultured with antigen-specific autologous lymphocytes. Supernatants were assayed for the costimulatory cytokine IL-1 beta which influences the outcome of DC:T cell interactions. The dendritic cells migrating from carcinogen-treated skin that failed to induce T cell proliferation were unable to produce IL-1 beta. This may account for the abrogation of DC function following exposure to chemical carcinogens and provides an explanation for the inability of DC to induce a protective immune response to carcinogen-induced tumours.

Said B, Ross MK, Salib T, Shank RC. **Modulation of DNA adduct formation by successive exposures of DNA to small and bulky chemical carcinogens**. Carcinogenesis 1995;16(12):3057-62.
The competition between N-methyl-N-nitrosourea (684935) (MNU) and the reactive derivatives of benzo (a)pyrene (50328), N-acetylaminofluorene (53963) (AAF), and aflatoxin-B1 (1162658) for guanine binding sites in a known DNA sequence was studied using a modified Maxam-Gilbert (MG) sequencing reaction and a DNA synthesis termination assay (DSTA). A 352 basepair DNA fragment of known sequence, and containing runs of guanine of seven and 13 bases, was prepared. In the MG analysis, the DNA was reacted with N-acetoxy-2-acetylaminofluorene (6098448) (AAAF) followed by (+)-r-7,t-8-dihydroxy-t-9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyr- ene (60268851) (BPDE), BPDE followed by AAAF, AAAF or BPDE followed by MNU, and MNU followed by AAAF or BPDE. In the DSTA, the DNA was reacted with MNU, AAAF, aflatoxin-B1-epoxide (42583460) (AFB1E), MNU followed by AAAF, and MNU followed by AFB1E. MG analysis showed that reaction of DNA with either BPDE or AAAF produced piperidine sensitive guanine sites. AAAF treatment produced DNA strand breaks, but the autoradiographic bands were not as intense as those produced by BPDE. Pretreatment of DNA with AAAF appeared to reduce the reaction of BPDE with DNA. BPDE pretreatment, however, did not inhibit damage by subsequent AAAF treatment. In fact, the combination of BPDE followed by AAAF appeared to be additive, as the loss of high molecular weight DNA was higher after BPDE plus AAAF than after BPDE alone. MNU methylation was greater than the arylation produced by either BPDE or AAAF; therefore, the effect of methylation on subsequent arylation could not be observed. Results of the DSTA showed that modification of DNA by pretreatment with MNU altered the binding of AAAF and AFB1E. MNU pretreatment reduced the intensity of the termination bands that result from C8-AAF-guanine or N7-AFB1-guanine adducts. The authors conclude that exposure of DNA to multiple carcinogens does not necessarily result in additive effects in adduct formation.

Sugimura T. **Overview of carcinogenic heterocyclic amines.** Mutat Res 1997;376(1-2):211-9.

Swenberg JA, La DK, Scheller NA, Wu KY. **Dose-response relationships for carcinogens**. Toxicol Lett 1995;82-83:751-6.
Recent advances in molecular dosimetry for carcinogenic agents were reviewed. Determination of dose response relationships for carcinogenic agents has been complicated by several factors including the kinetics of metabolic and detoxification pathways, the nature of the target macromolecule, DNA repair mechanisms, the mutational efficiency of DNA adducts, and the cellular proliferation rate. Several dose response relationships were described to illustrate difficulties inherent in molecular dosimetry. These included the formation of O6-dimethylguanine and N7-methylguanine following exposure to dimethylnitrosamine (62759), the formation of O4-ethylthymidine and O2-ethylthymidine following

exposure to diethylnitrosamine (55185), the formation of O6-methylguanine in response to N-methyl-(N-nitrosamino)-1-(3-pyridyl)-1-butanone (64091914), the formation of N-2,3-ethenoguanine by vinyl-chloride (75014) or vinyl-fluoride (75025), and the formation of 7-hydroxyethylguanine in response to ethylene-oxide (75218). The dose response relationship between formaldehyde (50000) and cancer induction was described as being one of the most nonlinear relationships recognized to date. The use of molecular dosimetry in risk assessment was described.

Tennant R. **Identifying carcinogens--a strategy for use of abbreviated models.** Toxicol Pathol 1997;25(2):240-1.

Weisburger JH. **A perspective on the history and significance of carcinogenic and mutagenic N-substituted aryl compounds in human health.** Mutat Res 1997;376(1-2):261-6.

Widel M, Dobrut M, Maka B, Lubecka B, Pluciennik A. **The radiosensitivity of human malignant melanomas evaluated by cytokinesis-block micronucleus assay.** Neoplasma 1997;44(2):109-16.
Cytokinesis-block micronucleus assay (CB-MNA) was applied for comparison of radiation sensitivity of 25 human malignant melanomas in primary culture. Cells obtained from tumor specimens were irradiated (0-4.Gy) on dishes, incubated with cytochalasin B (2 micrograms/ml) to block cytokinesis, stained in situ and micronuclei (MN) scored in binucleate cells (BNC). Proportions of BNC in nonirradiated controls after fixed time of incubation (96 h) ranged from 2.3 to 38% indicating great differences (C.V. = 74%) in proliferative activity among tumors evaluated. No correlation was observed between proliferative activity and susceptibility of cells to induction of MN by radiation. The great inter-tumor heterogeneity was observed in respect of radiation sensitivity expressed either as normalized (Net) frequency (Fq) of BNC with MN or as number of MN per BNC. Both endpoints differed widely at 2 Gy and 4 Gy as well (Net FqBNC with MN = 0.28-25.4% or 1.5-45% and MN/BNC = 0.004-0.309 or 0.013-0.593 respectively at 2 Gy and 4 Gy) with coefficients of variation ranging from 44 to 57%. Extreme difference in MN frequency was also observed between one primary tumor and its metastasis indicating intra-tumor heterogeneity. Our results suggest that CB-MNA may contribute some clinically useful information for discriminating tumors that will eventually respond to radiotherapy and those that will probably not. However, studies aimed at comparison of MN induction in vitro with clinical radioresponsiveness of malignant melanomas are urgently required.

Wu X, Shi H, Jiang H, Kemp B, Hong WK, Delclos GL, Spitz MR. **Associations between cytochrome P4502E1 genotype, mutagen sensitivity, cigarette smoking and susceptibility to lung cancer.** Carcinogenesis 1997;18(5):967-73.
BIOSIS COPYRIGHT: BIOL ABS. Cytochrome P4502E1 (CYP2E1) is involved in the metabolic activation of carcinogenic N-nitrosoamines. We therefore assessed the genotype frequencies of PstI or RsaI CYP2E1 restriction fragment length polymorphisms and another susceptibility marker, mutagen sensitivity, in 137 lung cancer cases (92 African American and 45 Mexican American) and 206 controls (114 African American and 92 Mexican American) identified in a molecular epidemiological study of lung cancer. The CYP2E1 c1/c1 genotype was found in 86.7% of Mexican American cases, 70.6% of Mexican American controls, 89.1% of African American cases and 86.8% of African American controls. By multivariate analysis, this genotype was found to be associated with a 14.0-fold increased risk of

lung cancer in Mexican Americans but not in African Americans; a 9.9-fold increased risk of lung cancer in Mexican American former smokers, but not in non-smokers or current smokers; a 15-fold increased risk of lung cancer in Mexican American males, but not in females. Patients with the susceptible genotype appeared to have developed cancer at an earlier age and with lower cigarette pack-year of exposure than did patients with the c1/c2 or c2/c2 genotypes. Stratified analysis suggested a greater than multiplicative interaction between cigarette smoking and CYP2E1 c1/c1 genotype, although not statistically significant. The odds ratios (ORs) for the CYP2E1 c1/c1 genotype, cigarette smoking and both risk factors combined were 1.3, 6.7 and 16.3, respectively. The association between CYP2E1 c1/c1 genotype and pack-years of smoking followed the same pattern. The interaction between mutagen sensitivity and CYP2E1 c1/c1 genotype was especially strong in former smokers (the ORs for the CYP2E1 c1/c1 genotype, mutagen sensitivity and both risk factors combined were 3.9, 5.4 and 23.0, respectively). Therefore, the data suggest that individuals who lack a c2 allele might be at higher risk for developing lung cancer.

Wu Y, Barnabas N, Russo IH, Yang X, Russo J. **Microsatellite instability and loss of heterozygosity in chromosomes 9 and 16 in human breast epithelial cells transformed by chemical carcinogens.** Carcinogenesis 1997;18(5):1069-74.
BIOSIS COPYRIGHT: BIOL ABS. Microsatellite instability (MSI) and loss of heterozygosity (LOH) in chromosomes 9 and 16 have been reported in human breast cancers. In order to determine whether changes in these chromosomes play a role in the initiation and progression of this disease, we performed microsatellite polymorphism analyses in human breast epithelial cells (HBEC) transformed by chemical carcinogens, an in vitro system that recapitulates various stages of neoplastic transformation. In this experimental system we studied the mortal HBEC MCF-10M, immortal MCF-10F cells, derived from MCF-10M cells, and clones derived from MCF-10F cells treated with benzo(a)pyrene (B(a)P) (BP1 and BP1-E) and 7,12-dimethylbenz(a)anthracene (DMBA) (D3 and D3-1). The four clones of transformed cells were injected into severe combined immunodeficient (SCID) mice. Only BP1-E cells induced the formation of tumors, designated BP1E-Tp cells. These cells originated six additional tumors, designated BP1E-Tf no. 1 through Tf no. 6. Microsatellite analyses were carried out using five markers for chromosome 9 and 20 for chromosome 16. There was no evidence of MSI or LOH in clones BP1 and BP1E when compared with the MCF-10M and MCF-10F cells, whereas BP1E-Tp cells and Bp1E-Tf no. 1-Tf no. 6 tumors exhibited MSI at loci p23 and p21, and LOH at p21-22 of chromosome 9. They also exhibited MSI and LOH at multiple loci of both the short and long arms of chromosome 16, i.e. p13.13, p13.3, p12, q12.1, q12.2, q23 and q24, to which putative tumor suppressor genes have been localized. Clones D3 and D3-1 exhibited no genomic changes in chromosome 9, but did show MSI at locus q12.1 of chromosome 16 using marker D16S285. Although the cells treated with DMBA expressed early phenotypes of neoplastic transformation, they were not tumorigenic, and also manifested fewer changes than the tumorigenic BP1E-Tp cells and the tumors BP1E-Tf. The changes in chromosomes 9 and 16 observed in these latter ones indicated an association with the expression of tumorigenesis, which represents a late event in the progression of the neoplastic transformation of HBEC. Of interest was the observation that HBEC transformed by chemical carcinogens in vitro express genomic changes similar to those found in spontaneous breast carcinomas.

CYTOTOXICITY

Gomez LA, Alekseev AE, Aleksandrova LA, Brady PA, Terzic A. **Use of the MTT assay in adult ventricular cardiomyocytes to assess viability: effects of adenosine and potassium on cellular survival.** J Mol Cell Cardiol 1997;29(4):1255-66.

Lopez DC, Jimenez A, Terencio J, Marin A, Bello J. **[Evaluation of cytotoxic potential of surfactants with MTT assay in different cell lines]**. Rev Toxicol 1996;13(1):13-9. (Spa)

Thornback-Lecoq S, Bettens JM, Zijlstra JA. **Correlation between skin irritation and cytotoxicity.** In Vitro Toxicol 1997;10(1):49-53.
BIOSIS COPYRIGHT: BIOL ABS. We are currently validating the use of cytotoxicity tests for predicting the possible skin irritating potential of various topical formulations. The cytotoxicity is determined in fibroblast cultures using the neutral red release (NRR) assay and the MIT test. For most products, the MTT test is more sensitive and detects cytotoxic effects at lower concentrations than the NRR test. The results of the cytotoxicity tests show a good correlation with the skin irritation Potential, which we measure on hairless guinea pigs. in general, a product is unlikely to be irritating if the IC50 is higher than 250 mg/ml in the NRR test, or higher than 125 mg/ml in the MTT assay, after 5 min exposure. The overall correlation between the cytotoxicity test and the skin irritation potential is good (r2 = 0.77 for 49 formulations tested). We found the cytotoxicity tests most useful as an aid for selecting the most promising among a series of similar formulations, thus significantly reducing the number of animal experiments. They can also be used for identifying the causative agent in an irritating complex mixture.

Vasanthakumari V, Nalini R, Devaraj H, Devaraj SN. **Cytotoxicity of methacrylonitrile**. Bull Environ Contam Toxicol 1997;59(2):274-8.
CBAC COPYRIGHT: CHEM ABS HepG2 cells were obtained and used in this study. Genotoxicity in HepG2 cells was investigated using the LSC based assay for unscheduled DNA synthesis. Quant. estns. were made with at least six plates for each concn. of methylacrylonitrile (MeAN). Maximal UDS activity was obsd. at a concn. of 20 nm/plate. The highest concn. tested, namely 40 nm/plate, yielded a modest depression of the UDS response. From the results, it can be concluded that MeAN may be a mutagen/carcinogen at lower doses, but at higher doses, it is cytotoxic.

DERMAL TOXICITY

Akimoto T, Aoyagi T, Minoshima J, Nagase Y. **Polymeric percutaneous drug penetration enhancer: synthesis and enhancing property of PEO/PDMS block copolymer with a cationic end group**. Proc Int Symp Controlled Release Bioact Mater 1997;24:471-2.
CBAC COPYRIGHT: CHEM ABS A PEO/polydimethylsiloxane block copolymer was prepd. by ring-opening polymn. of hexamethylcyclotrisiloxane with hydroxydimethylsilyl-terminated PEO, followed by termination with 3-chloropropyldimethylchlorosilane, conversion of the chloropropyl group to iodopropyl with NaI, and quaternization with Me2NEt. This polymer enhanced permeation of antipyrine and, to a lesser extent, indomethacin through rabbit skin in vitro. The PEO segment was necessary for

enhancement of penetration of hydrophilic drugs but not of hydrophobic drugs. The penetration enhancement was apparently due to an increase in partition into the adsorption layer of the stratum corneum, rather than to an increase in diffusion. The polymer showed a primary irritation index of 1.3 in the Draize test, and showed no corrosive effects.

Augustin C, Collombel C, Damour O. **Use of in vitro dermal equivalent and skin equivalent kits for evaluating cutaneous toxicity of cosmetic products.** In Vitro Toxicol 1997;10(1):23-31.
BIOSIS COPYRIGHT: BIOL ABS. Fourteen cosmetic products reflecting a range of irritancy levels were evaluated for cutaneous irritation potential using dermal equivalent (DE) and skin equivalent (SE) kits. Our two in vitro models are presented in 12 culture inserts (Transwell, Costar) allowing air-liquid interphase culture and topical application. Dermal equivalent includes a collagen-glycosaminoglycans-chitosan porous matrix populated by human normal fibroblasts and skin equivalent is realized by seeding keratinocytes onto the DE. The pure cosmetic products were applied in triplicate by topical application (10 mul) onto a surface delimited by a silicone assay ring placed onto DE and SE. After 24 h contact with the cosmetic products, the residual cellular viability was measured using a MIT test on treated and untreated tissues. The purpose of this preliminary validation study was to evaluate to what extent the in vitro results can predict in vivo skin irritation. Consequently, 11 cosmetic products with known Draize irritation classes were tested on DE. Ten of 11 were correlated if we considered only the irritancy potential prediction (irritant or nonirritant) but considering the binary correlation with three classes of irritation (irritant, slightly irritant, or nonirritant), 8 of 11 were correlated. Moreover, the parameters of validation were calculated. Second, three cosmetic products with known Draize primary irritation index (PDH) were tested both on DE and SE. The correlation of the in vitro MIT values to the in vivo data using a regression line was found to be r = 0.99 for DE and r = 0.99 for SE. These preliminary results are encouraging and suggest that the two models, DE kit and SE kit, could be used as in vivo alternative methods after a complete validation study involving the testing of the different chemical classes and various cosmetic forms.

Bando H, Mohri S, Yamashita F, Takakura Y, Hashida AM. **Effects of skin metabolism on percutaneous penetration of lipophilic drugs**. J Pharm Sci 1997;86(6):759-61.
CBAC COPYRIGHT: CHEM ABS Effects of skin metab. on percutaneous penetration of drugs with high lipophilicity were studied in vitro using rat skin pretreated with and without an esterase inhibitor, diisopropyl phosphorofluoridate [also known as diisopropyl fluorophosphate (DFP)]. Without DFP, about 96% of the total penetrated amt. appeared as metabolized p-hydroxybenzoic acid in the receptor fluid after application of butylparaben, whereas about 30% penetrated as intact form after application of propylparaben. On the other hand, metabolized p-hydroxybenzoic acid was not detected in the receptor fluid.

Bando H, Sahashi M, Yamashita F, Takakura Y, Hashida M. **In vivo evaluation of acyclovir prodrug penetration and metabolism through rat skin using a diffusion/bioconversion model**. Pharm Res 1997 Jan;14:56-62.
IPA COPYRIGHT: ASHP To study the in vivo penetration of prodrugs that are metabolized in the skin, the penetration profiles of model radiolabeled valerate, isovalerate, and pivarate prodrugs of acyclovir, with similar lipophilicity but different enzyme affinity, through rat skin were evaluated using a 2 layer

skin diffusion/bioconversion model; drug concentrations were measured in urine. Although total penetration amounts were similar for the 3 prodrugs, the ratio of intact prodrug to total penetration amount differed significantly. In addition, the excretion and absorption profiles were also very different for each prodrug. Enzymatic hydrolysis rate constants calculated under in vivo conditions were considerably larger than those obtained in other skin homogenate and in vitro penetration experiments.

Baynes RE, Halling KB, Riviere JE. **The influence of diethyl-m-toluamide (DEET) on the percutaneous absorption of permethrin and carbaryl.** Toxicol Appl Pharmacol 1997;144(2):332-9.
BIOSIS COPYRIGHT: BIOL ABS. Simultaneous exposure to DEET and permethrin was recently proposed to be associated with the Gulf War Syndrome. However, no studies have reported the percutaneous absorption of DEET and permethrin when applied simultaneously to the skin as a mixture, the relevant route of exposure in the Persian Gulf. The present study quantitates percutaneous absorption of DEET and permethrin after coadministration to rodent and pig skin in vitro. Dosing solutions were also prepared with either acetone, dimethyl sulfoxide (DMSO), or ethanol to compare vehicle effects on percutaneous absorption of permethrin and DEET. The influence of DEET on carbaryl absorption and dermal disposition was also assessed in pig studies to statistically demonstrate DEET effects in acetone or DMSO and different solvent concentrations. Topical application of permethrin + DEET resulted in absorption of DEET (1-20% dose), but no permethrin. Permethrin (1.2-1.7% dose) was detected only when mouse skin was dosed solely with permethrin, a finding suggesting that DEET decreased permethrin absorption. DEET also inhibited carbaryl absorption in acetone mixtures, but had no effect on DMSO mixtures. Irrespective of solvent, DEET did not enhance carbaryl penetration into skin. For DEET, absorption was greater in mouse skin (10.7-20.6% dose) than in rat skin (1.1-5.2% dose) and pig skin (2.8% dose). The extent of DEET absorption was greater with DMSO and acetone than with ethanol in rat and mouse skin. These studies support DEET, but not permethrin or carbaryl, as having sufficient systemic exposure to potentially cause signs of toxicity when simultaneously applied with pesticides. Furthermore, these studies demonstrated that DEET does not necessarily enhance dermal absorption of all toxicants as was originally hypothesized.

Beckley-Kartey SA, Hotchkiss SA, Capel M. **Comparative in vitro skin absorption and metabolism of coumarin (1,2-benzopyrone) in human, rat, and mouse.** Toxicol Appl Pharmacol 1997;145(1):34-42.
The in vitro percutaneous absorption and skin metabolism of coumarin (1,2-benzopyrone) was studied in metabolically viable human, rat (F344), and mouse (CD1 and DBA/2) skin. Following application of [14C]coumarin (3.7 microg/cm2; 0.02% in ethanol) to unoccluded skin in flow-through diffusion cells of a skin absorption model (SAM), the absorption through the skin into the receptor fluid at 72 hr was rapid and extensive in all species, reaching (mean +/- SD) 50.4 + 9.1% of the applied dose in human, 51.3 +/- 7.3% in rat, and 44.9 +/- 13.5% in mouse. When the skin was occluded immediately after exposure, the extent of absorption at 72 hr was enhanced in all species. At 72 hr, substantial amounts of [14C]coumarin were found in unoccluded mouse skin (31.7 +/- 13.6%), with less in human (10.2 +/- 6.5%) and rat (12.7 +/- 5.0%) tissue. When occluded, the skin residues at 72 hr were 10.4 +/- 11.7% (mouse), 8.5 +/- 3.9% (human), and 11.9 +/- 7.5% (rat). The absorption of coumarin through rat skin into the receptor fluid over 72 hr was linearly related to the applied dose (r2 = 0.998 unoccluded skin; r2 = 0.999 occluded skin) over the dose range 3.7 to 378.7 microg/cm2. The nature and extent of cutaneous

metabolism was studied following (i) topical application for 24 hr to human, rat, and mouse skin in the SAM system; (ii) incubation at 37 degrees C for up to 6 hr with human, rat, and mouse whole skin homogenates; and (iii) incubation at 37 degrees C for up to 24 hr with freshly isolated and cultured human epidermal keratinocytes. HPLC and GCMS analyses of skin extracts and receptor fluid confirmed that, in all three species, only the parent compound, coumarin, was present at all times from 10 min to 24 hr. These data indicate that topically applied coumarin is rapidly and extensively absorbed through human, rat, and mouse skin, and that the compound remains metabolically unchanged during absorption. These observations may have implications for the safe and effective use of coumarin in consumer products which come into contact with the skin and as a topical therapeutic agent.

Bettero A, Semenzato A. **[Evaluation of the irritancy and corrosiveness of surfactants and detergents by the B2A-P test]**. Cosmet News 1007;20,(112):22-3. (Ita)
CBAC COPYRIGHT: CHEM ABS A bioanalytic test for the evaluation of the toxic potential of ingredients and products made for the contact with skin and mucous membranes. The high resoln. of the test gives accurate data on the irritation power caused by the corrosive potential of the natural dose depending on surfactants and cleansing agents.

Bhatia KS, Gao S, Singh J. **Effect of penetration enhancers and iontophoresis on the FT-IR spectrometry and LHRH permeability through porcine skin**. J Controlled Release 1997 (Jul 7);47:81-9.
IPA COPYRIGHT: ASHP To investigate the effects of enhancers and iontophoresis on the in vitro permeability of gonadorelin (luteinizing hormone releasing hormone; LHRH) through porcine epidermis as well as to evaluate biophysical changes in the stratus corneum lipids, porcine skin was pretreated with either ethyl alcohol (ethanol), 10% oleic acid with ethyl alcohol, 10% oleic acid in propylene glycol, propylene glycol, or nothing and permeability of gonadorelin was evaluated passively and with iontophoresis; stratum corneum changes were observed using Fourier transform infrared spectrometry (FT-IR). All enhancers with the exception of propylene glycol alone increased permeability. Iontophoresis further increased permeability through the enhancers, showing synergy.

Bhatia KS, Gao S, Freeman TP, Singh J. **Effect of penetration enhancers and iontophoresis on the ultrastructure and cholecystokinin-8 permeability through porcine skin**. J Pharm Sci 1997;86 (9):1011-5.
CBAC COPYRIGHT: CHEM ABS The present study explores the effect of chem. penetration enhancers and iontophoresis on the in vitro permeability of cholecystokinin-8 (CCK-8) through porcine epidermis and on the ultrastructural changes in stratum corneum as obsd. by transmission electron microscopy (TEM). Enhancer [i.e., EtOH, and 10% oleic acid in combination with EtOH (OA/EtOH)] pretreatment significantly increased the permeability.

Davenport V, Morris JF, Chu AC. **Immunologic protection afforded by sunscreens in vitro**. J Invest Dermatol 1997;108(6):859-63.
CBAC COPYRIGHT: CHEM ABS Several studies have suggested a lack of correlation between sunscreen sun protection factor and protection of the skin immune system, potentially allowing greater damage to the skin by removing the natural protective erythemal response to sun exposure. Despite this,

routine testing of immune protection afforded by sunscreens is not performed by industry. Current lab. methods for investigating the efficacy of sunscreen protection of epidermal immune function use the induction of contact hypersensitivity or epidermal cell alloantigen presentation. Animal models, cell culture systems, and in vivo human studies are commonly employed, but all these systems have significant drawbacks for use in routine testing. The purpose of this study was to develop an in vitro system for testing the immunol. protection afforded by sunscreens in human skin. Five test sunscreens plus a vehicle control were tested in a blind fashion for their in vitro level of immune protection. Creams were applied in a std. manner to human whole skin explants and were irradiated over a range of physiol. doses using an Oriel solar simulator. A mixed epidermal lymphocyte reaction was used to quantify epidermal alloantigen-presenting capacity, in the presence or absence of test cream, for 5 explants. All the test sunscreens protected beyond their designated sun protection factors, whereas the vehicle conferred no protection. The explant-mixed epidermal lymphocyte reaction system gave consistent, reproducible results and may prove useful for the allocation of an immune protection factor to all sunscreens.

Dupuis L, Manfait M, Serpier H, Capon F, Kalis B. **Effects of ions on the hydrating potency of urea: study using ex vivo pigskin**. Int J Cosmet Sci 1997;19(1):37-44. (Fre)
CBAC COPYRIGHT: CHEM ABS This study deals with the influence of ions (NaCl and MgSO4) in a W/O emulsion contg. 10% urea. Moisturization kinetics are assessed by corneometry on pig skin ex vivo. The formula's influence on urea penetration is measured by IR spectrometry with an ATR device and stripping method. Corneometry and spectroscopy were chosen to record simultaneously the hydration levels and urea localization into superficial cell layers. Urea crystn. after evapn. of emulsions and aq. solns. is described. Results show that urea does not hydrate nor penetrate when applied to the skin through an aq. gel. In a W/O emulsion, sodium chloride increases the ability of urea to moisturize without improving penetration. In vitro urea crystn. is disturbed by sodium chloride or magnesium sulfate for solns. and emulsions. This stabilization by ions is correlated with good moisturization values. The stabilization of urea in the solute sate provided by ions increases its water epidermal binding capacity without enhancing penetration.

Fartasch M. **Ultrastructure of the epidermal barrier after irritation.** Microsc Res Tech 1997;37 (3):193-9.
The stratum corneum (SC) controls the diffusion and penetration of chemical substances and drugs into and through the skin. Surprisingly, knowledge of the SC structure and reaction to the various irritants is still poorly understood. Routine transmission electron microscopy has not been effective in demonstrating the epidermal lipids (EL) of SC which are believed to morphologically represent the water permeability barrier. To gain a better understanding of the interaction of chemically different irritants with the SC, we investigated the ultrastructural changes of epidermal lipids resulting from the topical application of sodium dodecyl sulfate (SDS 0.5% and 1% w/v) and absolute acetone. The disturbance of barrier function by these irritants was determined by the increase of transepidermal water loss (TEWL). Punch biopsies from the treated sites showed a maximum increase of TEWL. To visualize the EL which derive from lamellar body (LB) lipids (sheets), we used a special fixation method utilizing 0.5% ruthenium tetroxide/0.25% KFe(CN)6 as the postfixative. The 0.5% SDS caused cell damage to the nucleated cells of the epidermis with disturbance of LB lipid extrusion and the transformation into

the lipid bilayers. However, the upper portions of SC displayed intact intercellular lipid layers. With the acetone treatment, the EL lamellae showed disruption and loss of cohesion between the lamellae at all levels of the SC. The more polar LB lipids appeared more resistant to acetone. The results of this study suggest that different irritants induce distinct and characteristic alterations to reflect the specific interaction with the epidermal permeability barrier.

Fujii M, Yamanouchi S, Nagakura K, Takeda Y, Matsumoto M. **[Enhancement effect of menthoxypropandiol and menthol on the penetration of indomethacin through Yucatan micropig skin in vitro]**. Drug Deliv Syst 1997;12(2):127-31. (Jpn)

Godwin DA, Michniak BB, Creek KE. **Evaluation of transdermal penetration enhancers using a novel skin alternative**. J Pharm Sci 1997; 86(9):1001-5.

Griffiths HA, Wilhelm KP, Robinson MK, Wang XM, McFadden J, York M, Basketter DA. **Interlaboratory evaluation of a human patch test for the identification of skin irritation potential/ hazard.** Food Chem Toxicol 1997;35(2):255-60.
The human 4 hr patch test provides an opportunity to identify substances with significant skin irritation potential without recourse to the use of animals. To demonstrate the validity of the method it must be relevant and reliable. It is self-evident that the method is relevant to the identification of skin irritation hazards to humans. However, it is essential that the results be reproducible. This paper presents data on a number of substances tested by different laboratories. Eight substances were tested by two or more laboratories and the data compared with a standard positive control, 20% sodium dodecyl sulfate. In almost all cases, the outcome of this comparison was identical. Thus, despite the fact that there is known variability among human subjects in terms of skin reactivity to irritants, this simple method showed good reproducibility for the classification of acute skin irritation potential. Therefore, it is argued that this human 4-hr patch test is a valid alternative to the equivalent rabbit test for the assessment of skin irritation hazard to humans.

Gysler A, Lange K, Korting HC, Schafer-Korting M. **Prednicarbate biotransformation in human foreskin keratinocytes and fibroblasts.** Pharm Res 1997;14(6):793-7.
PURPOSE: Evaluation of skin layer-specific prednicarbate (PC) biotransformation, possibly explaining the improved benefit/risk ratio of this topical corticosteroid in atopic dermatitis (1,2). METHODS: Metabolism of PC in keratinocyte and fibroblast monolayers derived from human juvenile foreskin was evaluated. Drug concentration was determined by HPLC/UV-absorption. Accompanying cell viability tests (MTT-tests) were performed to exclude toxic drug effects. RESULTS: Keratinocytes hydrolyzed the double ester PC (2.5 x 10(-5) M) at position 21 to the monoester prednisolone 17-ethylcarbonate (P17EC) which nonenzymatically transformed to prednisolone 21-ethylcarbonate (P21EC). This metabolite was enzymatically cleaved to prednisolone (PD), the main biotransformation product at 24 hours. Fibroblasts, however, showed a distinctively lower enzyme activity. Both, PC and P17EC (or rather P21EC) were hydrolyzed to a minor extent only. The biotransformation pathway, however, was the same. When P17EC was added separately, it transformed to P21EC and again was cleaved by keratinocytes to a much higher extent. Despite of the rather high glucocorticoid concentration MTT-tests proved a non-disturbed cell viability and proliferation rate. CONCLUSIONS: Extrapolating our results

to the in-vivo situation, topically applied PC may be metabolized by epidermal cells during skin penetration. A complex mixture of compounds reaches the dermis, whose fibroblasts are barely able to metabolize the steroids. Since skin atrophy is less pronounced with PC as compared to conventional halogenated glucocorticoids, less potent PC metabolites appear to be the dominant species in the dermis.

Hikima T, Tojo K. **Binding of prednisolone and its ester prodrugs in the skin**. Pharm Res 1997;14:197-202.
IPA COPYRIGHT: ASHP The skin binding and penetration of prednisolone and various ester prodrugs of prednisolone with different alkyl side chain lengths, ranging from shortest to longest in order in the prodrugs senesyonate, geranate, farnesylate, and geranylgeranate, were studied in vitro in hairless Hr-/Kud strain mouse skin. The binding capacity for prednisolone and senesyonate was homogeneous in the viable skin while that of geranate and farnesylate gradually increased as the distance from the skin surface increased. The steady state penetration rate of the prednisolone ester prodrugs decreased with increasing alkyl chain length, and there was little penetration of geranylgeranate into the skin. The fraction of metabolite to esters that penetrated across skin increased with increasing alkyl chain length.

Jiang R, Roberts MS, Prankerd RJ, Benson AH. **Percutaneous absorption of sunscreen agents from liquid paraffin: self-association of octyl salicylate and effects on skin flux**. J Pharm Sci 1997;86 (7):791-6.
CBAC COPYRIGHT: CHEM ABS This study provides an investigation of the availability of octyl salicylate (OS), a common sunscreen, from liq. paraffin and the effect of OS on skin permeability. A model membrane system to isolate the vehicle effect from membrane permeability was developed. Partitioning of OS between liq. paraffin and aq. receptor phases was conducted. Partition coeffs. increased with increase in OS concn. A range of OS concns. in liq. paraffin was diffused across human epidermis and synthetic membranes into 4% bovine serum albumin in phosphate-buffered saline and 50% ethanol. Absorption profiles of OS obtained from silicone and low-d. polyethylene membranes were similar to each other but higher than for the high-d. polyethylene (HDPE) membrane and human epidermis. The steady state fluxes and apparent permeability coeffs. (Kp') obtained from the diffusion studies showed the same trends with all membranes, except for the HDPE membrane which showed greater increase in flux and Kp' at concns. above 30%. IR spectra showed that several bands of OS were shifted with concns., and the mol. models further suggested that the main contribution to the self-assocn. is from non-1,4 van der Waals interactions.

Kawasaki Y, Quan D, Sakamoto K, Maibach HI. **Electron resonance studies on the influence of anionic surfactants on human skin.** Dermatology 1997;194(3):238-42.
BACKGROUND: When skin is exposed to chemicals, raw materials interact with the lipid structure of the stratum corneum. At least two types of disorders can be distinguished--that of alkyl chains inside one lipid bilayer and that of lipid layer arrangement. Electron spin resonance (ESR) spectroscopy of a nitroxide spin label is a valuable method in the study of biological membranes. OBJECTIVE: These experiments define the effect of anionic surfactants on the lipid bilayer of human stratum corneum. METHODS: 5-Doxyl stearic acid (5-DSA) was used as the spin label. Sodium lauryl sulfate (SLS) and sodium lauroyl-L-glutamate (SLG) were the anionic surfactants studied. ESR spectrum measurements of surfactant-treated stratum corneum were performed and order parameters calculated. RESULTS: 1% of

SLS leads to an obvious change in ESR spectra--from strongly to weakly immobilized spectra. The molecular motion of spin labels (5-DSA) in SLS-treated stratum corneum is different from that of spin labels in the untreated stratum corneum. The ESR spectra suggest that SLS affects the spin label binding to the lipid membrane and causes an increase in the mobility of bilayers. On the other hand, there were minimal changes in ESR spectra of 1% of SLG-treated stratum corneum. An increase in fluidity of skin lipid bilayers suggests a decrease in the skin barrier function. CONCLUSION: ESR may provide a facile and robust method to define the subclinical irritancy potential of anionic surfactants and other materials.

Kietzmann M, Blume B. **Percutaneous absorption of betamethasone from different formulations using the isolated perfused bovine udder**. In Vitro Toxicol 1997;10(1):11-5.

Lewis D, Paulo M, Faustino E, Farinha A. **In vitro comparative studies of transdermal nicotine delivery systems**. Int J Pharm 1997;148(2):177-89.
CBAC COPYRIGHT: CHEM ABS In vitro release rates of nicotine from 3 transdermal systems available in the Portuguese and Spanish markets were compared in vitro by methods based on proposed USP release tests and assay, and using Franz diffusion cells with membranes of 'full-thickness' porcine ear or human breast skin. No significant differences were found between the release profiles obtained by the different release test methods for each device and it may be possible and desirable to further standardize testing recommendations. Total nicotine content could not be detd. for 1 device at modest agitation speeds by methanol extn. Some simple approaches to comparing animal models and release tests with human skin expts. in vitro are discussed in relation to potential applications to quality control of TDS.

Millership JS, Collier PS. **Topical administration of racemic ibuprofen**. Chirality 1997;9(3):313-6.
CBAC COPYRIGHT: CHEM ABS In vitro expts. to investigate possible stereoselective aspects of the topical administration of ibuprofen have been conducted. Incubation of ibuprofen with rat skin homogenates in the presence of CoA, ATP, and magnesium provided no evidence for the formation of ibuprofenyl CoA (the initial intermediate in the metabolic inversion of [R]- to [S]-ibuprofen). Similar incubation studies gave no indication of a change in the enantiomeric ratios of ibuprofen over the time course of the expts. Percutaneous penetration studies of ibuprofen gel through porcine skin indicated that the ibuprofen enantiomer levels in the reservoir solns. were consistent with racemic ibuprofen having traversed the skin with no metabolic inversion. These results suggest that, in the models studied, skin metab. does not result in the chiral inversion of (R)- to (S)-ibuprofen and that the topical administration of ibuprofen will result in the delivery of 50% isomeric ballast.

Monteiro-Riviere NA, Inman AO. **Ultrastructural characterization of sulfur mustard-induced vesication in isolated perfused porcine skin**. Microsc Res Tech 1997;37(3):229-41.
CBAC COPYRIGHT: CHEM ABS For this study, 200 muL of either 10.0, 5.0, 2.5, 1.25, 0.50, or 0.20 mg/mL of bis(2-chloroethyl) sulfide (HD) in ethanol or ethanol control was topically applied to a 5.0 cm2 dosing area of the isolated perfused porcine skin flap (IPPSF) and perfused for 8 h with recirculating media. HD dermatotoxicity was assessed in the flap by cumulative glucose utilization (CGU), vascular resistance (VR), light microscopy (LM), SEM, and TEM. HD produced a statistically significant dose relation for gross blisters and microvesicles. The HD-treated IPPSFs were also

characterized by a decrease in CGU and an increase in VR. Light microscopic changes included mild intracellular and slight intercellular epidermal edema, multifocal epidermal-dermal sepn., and dark basal cells. Ultrastructural alterations consisted of cytoplasmic vacuoles, pyknotic basal cells, nucleolar segregation, and epidermal-dermal sepn. occurring between the lamina lucida and lamina densa of the basement membrane. The severity of these changes increased in a dose-dependent manner. Morphol., the IPPSF appeared similar to human skin exposed to HD with the formation of macroscopic blisters and microscopic vesicles. In conclusion, the IPPSF appears to be an appropriate in vitro model with which to study the pathogenesis of vesicant-induced toxicity.

Monteiro-Riviere NA, Inman AO, Snider TH, Blank JA, Hobson DW. **Comparison of an in vitro skin model to normal human skin for dermatological research.** Microsc Res Tech 1997;37(3):172-9.
EpiDerm, an in vitro human skin equivalent (HSE), was compared to normal human breast skin (NHS) to morphologically and biochemically assess its feasibility for dermatological research. Intralot and interlot variability was studied in day 0, 1, 2, and 3 in vitro cultures and in day 0, 3, 5, and 7 NHS. For NHS, light microscopy (LM) at day 0 showed stratified epidermis which exhibited an increase in vacuoles and dark basal cells as storage increased to 3, 5, and 7 days. Transmission electron microscopy (TEM) revealed typical organelles in the epidermis and a convoluted basement membrane at day 0. With increased storage, vacuoles and paranuclear clefts became numerous, necrosis increased, tonofilaments became less organized, and overall cellular integrity decreased. Biochemical data showed consistent MTT and glucose utilization (GU) through day 5, while lactate production decreased to 75% by day 3. By LM, day 0 HSE consisted of a thick, compact, stratum corneum that sent projections between the stratum granulosum cells. By TEM, the configuration organization, differentiation, distribution, and frequency of the organelles differed slightly from NHS. In addition, the basement membrane of the HSE was not completely differentiated, and the dermis was thin and acellular. Although day 1 and 2 cultures showed little change, day 3 exhibited an overall degeneration. Biochemical analysis showed GU and the lactate production decreased through day 3. In conclusion, the EpiDerm HSE, although exhibiting slight differences, was morphologically and biochemically similar to normal human epidermis and may be a valuable model in assessing the toxicology, metabolism, or of nonvesicating compounds.

Monteiro-Riviere NA, Riviere J. **The pig as a model for cutaneous pharmacology and toxicology research**. Adv Swine Biomed Res 1996;2:425-58.

Nakai JS, Chu I, Li-Muller A, Aucoin R. **Effect of environmental conditions on the penetration of benzene through human skin**. J Toxicol Environ Health 1997;51(5):447-62.
CBAC COPYRIGHT: CHEM ABS The in vitro penetration of [14C]benzene through freshly prepd. human skin was examd. under a variety of skin conditions assocd. with swimming and bathing. The permeability coeff. of 0.14 cm/h under std. conditions at 26.degree.C was found to increase to 0.26 cm/h at 50.degree.C and decrease to 0.10 cm/h at 15.degree.C. Storage of the skin at -20.degree.C did not affect the penetration of benzene. Application of baby oil, moisturizer, or insect repellent to the skin before exposure under std. conditions did not affect the flux of benzene, but a significant increase was obsd. when the skin was pretreated with sunscreen (permeability coeff. 0.24 cm/h). These results suggest that risk assessment or exposure modeling for benzene and other environmental contaminants should account for appropriate changes in the environmental conditions when considering the dermal route of

exposure.

Nakajima N, Kakubari I, Uruno A, Kawakami J, Takayasu T, Yamauchi H, Takayama S. **Effects of solvents on the skin penetration of formoterol fumarate**. Proc Int Symp Controlled Release Bioact Mater 1997;24:687-8.
CBAC COPYRIGHT: CHEM ABS A system consisting of l-menthol/N-methyl-2-pyrrolidone was an effective enhancer of the skin penetration of formoterol fumarate, suggesting that it has potential for transdermal delivery of formoterol.

Pellett MA, Roberts MS, Hadgraft J. **Supersaturated solutions evaluated with an in vitro stratum corneum tape stripping technique**. Int J Pharm 1997;151(1):91-8.
CBAC COPYRIGHT: CHEM ABS The flux of a compd. across a membrane from any formulation, whether it contains penetration enhancers or not, is limited by its satd. soly. in the vehicle. Under such conditions the concn. of the permeant in the outer layers of the stratum is also satd. Consequently, when the permeation of a drug from a supersatd. soln. leads to enhanced penetration, the.

Pittermann W, Jackwerth B, Schmitt M. **The isolated perfused bovine udder skin model: a new in vitro model for the assessment of skin penetration and irritation.** In Vitro Toxicol 1997;10(1):17-21.
BIOSIS COPYRIGHT: BIOL ABS. The isolated perfused bovine udder skin (BUS) model was developed for studies concerning percutaneous absorption of pharmaceutical substances (Kietzmann et al., 1993). Additionally this in vitro method provides new possibilities for the assessment of the skin irritation potential. The comparison of the prostaglandin E2-concentrations and MIT values of untreated control sites obtained simultaneously to treated skin sites showed the unimpaired vitality during the perfusion period of 20 individual bovine udder studies for assessment of skin irritation. Furthermore the results reveal no seasonal influences on the suitability of the organs perfused. Out of a number of tests concerning body care ingredients and formulations the results of two surfactants widely used were selected. Sodium lauryl sulfate (SLS) and alkyl polyglycoside (APG) were applied on the udder skin with low concentrations of 3% and 10% active substance, respectively, under occlusive conditions for 1 h and 5 h. The biological effects in the epidermal and dermal layers were characterized by means of prostaglandin E2 assay and methyl tetrazolium salt dye conversion. The two surfactants could be clearly differentiated. The skin mildness of APG concerning cytotoxicity and synthesis of eicosanoids (e.g. irritancy after exposure up to 5 h) was proven in conformity with human studies.

Ray TA, Mackerer CR, Blackburn GR, Goldstein LS. **Manufactured gas plant tar residue dermal exposure assessment as part of a site remediation program**. Polycyclic Aromat Compd 1996;10(1-4):351-8.
CBAC COPYRIGHT: CHEM ABS Solid-liq. partitioning expts. were designed to compare the sorptive properties of petroleum coke and activated charcoal with manufd. gas plant (MGP) tar residues. In addn., in vitro dermal penetration studies using human skin sections were carried out with a neat MGP tar from an MGP site and petroleum coke-MGP tar mixts. Dermal expts. were conducted with neat MGP tar, 1:1, and 1:9 coke-MGP tar mixts. over 144 h under infinite dose conditions (25 mg/cm2 skin surface) to det. the relative permeation of MGP tar components through human skin. The neat MGP tar and coke-MGP tar mixts. were fortified with 3H-benzo[a]pyrene prior to the dermal expt. to facilitate

measurement of skin permeation. Results from the partitioning expts. showed petroleum coke to have 1/3 the sorptive capacity of activated charcoal. In comparison to neat MGP tar, the dermal bioavailability of the coke-MGP tar polynuclear arom. compds. (PAC) decreased by a factor of 6 for the 1:1 mixt. and by a factor of >500 for the 1:9 mixt. The data can be used to est. the dermally absorbed dose (DAD) and the chronic systemic health effect risks assocd. with potential dermal exposure to MGP tars at former MGP sites and to det. the required admixing ratio of coke and MGP tar to achieve acceptable risks.

Reifenrath WG, Kemppainen B, Palmer WG. **An in vitro pig skin model for predicting human skin penetration and irritation potential**. Adv Swine Biomed Res 1996;2:459-74.

Santi P, Colombo P, Bettini R, Catellani PL, Volpato NM, et al. **Drug reservoir composition and transport of salmon calcitonin in transdermal iontophoresis**. Pharm Res 1997;14(1):63-6.
IPA COPYRIGHT: ASHP Anodal iontophoretic transdermal administration of 100 IU of calcitonin salmon through shaved rabbit skin was studied in vivo at pH values 4.2 and 7.4 using 2 types of drug reservoirs applied to the skin that either consisted of a thin dry disc containing 100 IU of the drug, which was covered with a cellulose pad and wetted with a buffer solution, or consisted of a cellulose pad wetted with 100 IU of the drug dissolved in a buffer solution. Transdermal iontophoresis of calcitonin salmon elicited a decrease in the serum calcium level, whereas, in the absence of electric current, no significant decrease was observed. When using the reservoir prepared from drug solution, iontophoresis was more effective at pH 4.2 than at pH 7.4. When using the reservoir prepared from the dry disc, similar kinetics and extent of drug effect were observed at both pH values. Also, the dry disc reservoir concentrated calcitonin salmon next to the skin.

Santoyo S, Arellano A, Ygartua P, Martin C. **In vitro percutaneous absorption of piroxicam through synthetic membranes and abdominal rat skin**. Pharm Acta Helv 1996;71(2):141-6.
IPA COPYRIGHT: ASHP The in vitro release of piroxicam from carbomer 940 gels and its penetration through isopropyl myristate impregnated membranes and abdominal rat skin were investigated, and attempts were made to relate the differences in the release rate with physicochemical properties of the drug and the vehicle. Piroxicam was released from the topical gel formulations and diffused through skin. It was suggested that although piroxicam flux across abdominal rat skin was lower than through isopropyl myristate membranes, this kind of membrane can be used in preliminary screening among the different piroxicam formulations.

Schmalz G, Arenholt-Bindslev D, Hiller KA, Schweikl H. **Epithelium-fibroblast co-culture for assessing mucosal irritancy of metals used in dentistry.** Eur J Oral Sci 1997;105(1):86-91.
No valid animal or in vitro model exists to assess the potential mucosal irritancy of dental materials. However, recently, a commercially available model system based on a recombined co-culture of human fibroblasts and human epithelial cells has been introduced for evaluating the time-dependent irritancy of cosmetic products. Cell viability and prostaglandin E2 (PGE2) release from the cells were used as markers for the irritative potential of test materials. The objective of the present study was to evaluate the suitability of this model for monitoring the irritative potential of metals and cast alloys used in

dentistry. The human fibroblast-keratinocyte co-cultures were exposed to test specimens fabricated from copper, zinc, palladium, nickel, tin, cobalt, indium, a high noble cast alloy, and from a dental ceramic. Cell survival rates decreased after exposure to copper.

Simion FA. **Human in vivo methods for assessing the irritation potential of cleansing systems**. Surfactant Sci Ser 1997;68:519-32.
CBAC COPYRIGHT: CHEM ABS A review with 47 refs. Closed patch testing, exaggerated use tests, assessment of irritation potential of surfactants on damaged skin, assessing the initial effects of surfactants on skin, methods to asses sensory irritation from surfactants, assessing skin dryness, and strategies for selecting test methods are discussed.

Singh R, Vyas SP. **Selective drug delivery through and within skin using liposomes**. Indian J Pharm Sci 1996;58(1):9-17.
IPA COPYRIGHT: ASHP A review of selective drug delivery through skin using liposomes is presented, including cutaneous and percutaneous absorption, dermal drug delivery, liposomes as drug carriers, rationale and advantages of controlled drug delivery with liposomes, selective drug delivery through and within skin using liposomes, liposome skin interaction and toxicity, in vitro skin penetration studies, in vivo drug disposition studies, biocompatibility studies, mechanisms of topical liposomal drug delivery, and liposomes in transdermal drug delivery.

Staniek V, Misery L, Peguet-Navarro J, Abello J, Doutremepuich JD, Claudy A, Schmitt D. **Binding and in vitro modulation of human epidermal Langerhans cell functions by substance P**. Arch Dermatol Res 1997;289(5):285-91.

Sznitowska M. **Influence of ethanol on permeation behavior of the porous pathway in the stratum corneum**. Int J Pharm 1996 Jun 21;137:137-40.
IPA COPYRIGHT: ASHP To investigate the effects of ethyl alcohol (ethanol) on the percutaneous absorption of zwitterions, the absorption of baclofen in the presence of 0-95% ethyl alcohol (ethanol) was investigated in vitro. A slow time-dependent increase of penetration rate was observed for lower ethyl alcohol concentrations. At 0-70% ethyl alcohol, solubility of baclofen was the dominant factor influencing penetration rate. At 95%, ethyl alcohol increased the permeability coefficient of baclofen by a factor of 10.

Sznitowska M, Janicki S, Gos T. **Effect of sorption promoters on percutaneous permeation of a model zwitterion--baclofen**. Int J Pharm 1996 Jun 21;137:125-32.
IPA COPYRIGHT: ASHP To investigate the effects of absorption enhancers on the percutaneous penetration of zwitterions, the absorption of baclofen in the presence of dimethyl sulfoxide, urea, propylene glycol, sodium lauryl sulfate (sodium lauryl sulphate), 95% ethyl alcohol (ethanol), laurocapram (Azone), oleic acid, and oleic acid/propylene glycol system across excised human cadaver full-thickness skin was evaluated. No significant increase of penetration or skin accumulation of baclofen was observed when dimethyl sulfoxide, urea, propylene glycol, laurocapram, or oleic acid were used. The presence of sodium lauryl sulfate or oleic acid/propylene glycol resulted in high penetration

rates and uptake of baclofen but this effect was observable only after 30 h and was accompanied with signs of barrier damage.

Tanojo H, Geest AB, Bouwstra JA, Junginger HE, Bodde HE. **In vitro human skin barrier perturbation by oleic acid: thermal analysis and freeze fracture electron microscopy studies**. Thermochim Acta 1997;293(1-2):77-85.
CBAC COPYRIGHT: CHEM ABS This study aims to elucidate the working mechanism of oleic acid (OA) on isolated human stratum corneum (SC) sheets by using 2 in vitro techniques, DTA and freeze-fracture electron microscopy. DTA on SC after the application of OA in propylene glycol revealed significant changes in the thermal profiles of SC compared to that of the untreated SC. The changes occurred generally on the lipid phase transitions by both shifting the temps. to a lower degree and reducing the enthalpies of the transitions normally obsd. between 40.degree. and 90.degree.. Another newly obsd. change took place in the temp. range below 0.degree., referred to as the subzero region. The subzero transition of OA has profoundly influenced the subzero SC lipid transition (normally obsd. at around -10.degree.) by shifting it to a lower temp. The interesting observation was that the subzero transition of SC lipid and of OA became a single transition after the SC is heated to 120.degree., which indicates a close interaction between oleic acid and SC lipids. Electron micrographs obtained by freeze-fracture electron microscopy revealed the formation of a new structure in the intercellular lipid regions of SC in the presence of OA. Oleic acid acts as a skin penetration enhancer by forming together with SC lipid a new type of lipid domain which is responsible for the decreased capacity of skin barrier function after oleic acid treatment.

Tian W, MY, Yang YH, Zhu CE. **[Effect of penetration enhancers on in vitro permeability of ibuprofen through mouse skin]**. Chin J Pharmaceut 1996 Apr;27:161-3. (Chi)
IPA COPYRIGHT: ASHP To investigate the effects of penetration enhancers on the skin permeability of ibuprofen, ibuprofen with either laurocapram, urea, or polysorbate 80 (Tween 80) were evaluated for skin permeability using intact mouse skin. The enhancing effect of laurocapram was much greater than that of urea and polysorbate 80.

Touraille G, Stinchcomb A, Pirot F, Guy R, Bunge A, Marty J. **In vivo and in vitro percutaneous absorption of 4-cyanophenol (4CP) from soil. Predictive modeling and influence of soil loading**. Proc Int Symp Controlled Release Bioact Mater 1997;24:739-40.

Trotta M, Pattarino F, Gasco MR. **Influence of counter ions on the skin permeation of methotrexate from water-oil microemulsions**. Pharm Acta Helv 1996;71(2):135-40.
IPA COPYRIGHT: ASHP The influence on the penetration of methotrexate across intact hairless mouse skin of different counter ions added to water-oil microemulsions containing lecithin, as surfactant, and water-propylene glycol at different pH values, as internal phase, was studied; monooctyl phosphate, monodecyl phosphate, monodecyl glycerophosphate, taurodeoxycholate, dodecyl sulfate, and dioctyl sulfosuccinate were used as counter ions. At pH 4, the transport of methotrexate was enhanced by the counter ions and a marked increase in the flux of drug was measured when dodecyl sulfate and dioctyl sulfosuccinate were used. At pH 5, only a slight increase of the flux was observed. The increased permeation was attributed to the lipophilization of methotrexate as a consequence of ion pair formation.

Wagner C, Gobel S, Wohlrab W, Neubert R. **Vehicle-dependent biotin liberation and penetration into human skin**. Skin Pharmacol 1997;10(1):34-9.

Wester RC, Hartway T, Serranzana S, Maibach HI. **Human skin in vitro percutaneous absorption of gaseous ethylene oxide from fabric**. Food Chem Toxicol 1997;35(5):513-5.
CBAC COPYRIGHT: CHEM ABS Ethylene oxide, a colorless gas at ordinary room temp. and pressure, is widely used as a fumigant, coming in contact with clothing and human skin. It is genotoxic in somatic and germ cells. [1,2-14C]Ethylene oxide and fabric disks were sealed in a glass container; the fabric disks were then removed and placed on human skin mounted in glass diffusion cells. Percutaneous absorption was 1.3% of the dose when the fabric/skin surface was open to surrounding air, and increased to 46.0% when the surface was occluded with latex glove material. The absorption was rapid, occurring within the first 0-4 h assay period. Absorbed chem. was confirmed to be unchanged ethylene oxide in the receptor fluid. This study also serves as a model for exposure of fabric/skin to any potentially hazardous gas.

Wester RC, Quan D, Maibach HI. **In vitro percutaneous absorption of model compounds glyphosate and malathion from cotton fabric into and through human skin**. Food Chem Toxicol 1996;34 (8):731-5.

Wolf R, Raith K, Neubert R. **Separation and quantitation of glycolipids as penetration modifiers in human skin using high-performance liquid chromatography-mass spectrometry with electrospray ionization**. J Chromatogr, A 1997;766(1): 71-5.
CBAC COPYRIGHT: CHEM ABS A HPLC-mass spectrometry method is presented for the measurement of glycolipids used as modulators of the penetration of drugs into human skin. In methanol exts. from different skin layers a detection limit of 100-400 pg/mL could be achieved. A routine anal. procedure could be set up with good quantitation reliability (relative std. deviation 6.6%).

Wu P, Huang Y, Fang J, Tsai Y. **In vitro percutaneous absorption of captopril**. Int J Pharm 1997;148 (1):41-6.
CBAC COPYRIGHT: CHEM ABS Four available skin membranes (mouse, rat, rabbit, pig) and human skin were utilized to evaluate the in vitro penetration of captopril. The flux of captopril increased in the order of human = pig < rabbit < rat < mouse. The penetration rate of captopril through rabbit skin was optimal to evaluate the variation of formulations. Hence, the rabbit skin was picked out as a model membrane for in vitro penetration expts. of captopril. The flux of captopril was increased.

Yozomizo Y. **Effect of phosphatidylglycerol on the in vitro percutaneous drug penetration through the dorsal skin of guinea pigs, and analysis of the molecular mechanism, using (ATR-FTIR) spectroscopy**. Int J Pharm 1997;147(2):219-31.

ECOTOXICITY

Bearden AP, Schultz TW. **Structure-activity relationships for Pimephales and Tetrahymena: a mechanism of action approach.** Environ Toxicol Chem 1997;16(6):1311-7.
BIOSIS COPYRIGHT: BIOL ABS. The toxicity data of 74 chemicals tested in both the 96-h fathead minnow (Pimephales promelas) mortality assay and the 2-d Tetrahymena pyriformis (a protozoan) growth inhibition assay were evaluated using quantitative structure-activity relationships (QSARs). Each chemical was a priori assigned a mechanism of acute toxic action from either nonpolar narcosis, polar narcosis, weak acid respiratory uncoupling, soft electrophilicity, or proelectrophilicity. The polar narcotics were further split into a phenol group and an aniline group. The relationship between bioreactivity and the importance of penetration to the site of action in both systems was studied. Bioreactivity showed a trend to be inversely proportional to the value of the hydrophobicity term. The data were examined to investigate how different molecular descriptors modeled the mechanisms of action. Models were produced for nonpolar narcotics and anilines for both species with the 1-octanol/ water partition coefficient (log Kow) alone. Soft electrophiles were best predicted by the average acceptor superdelocalizability (Savn), whereas proelectrophiles were modeled by log Kow and Savn. The weak acid uncouplers modeled with either log Kow or log Kow plus the ionization constant (pKa) for Pimephales and Tetrahymena, respectively. Phenols yielded predictive models using a either a combination of log Kow with Savn or lowest unoccupied molecular orbital, for fathead minnow and protozoan, respectively.

Boyd EM, Meharg AA, Wright J, Killham K. **Assessment of toxicological interactions of benzene and its primary degradation products (catechol and phenol) using a lux-modified bacterial bioassay**. Environ Toxicol Chem 1997;16(5):849-56.
CBAC COPYRIGHT: CHEM ABS A bacterial bioassay has been developed to assess the relative toxicities of xenobiotics commonly found in contaminated soils, river waters, and ground waters. The assay utilized decline in luminescence of lux-marked Pseudomonas fluorescens on exposure to xenobiotics. Three principal environmental contaminants assocd. with benzene degrdn. were exposed to the luminescence-marked bacterial biosensor to assess their toxicity individually and in combination. Median effective concn. (EC50) values for decline in luminescence were detd. for benzene, catechol, and phenol and were found to be 39.9, 0.77, and 458.6 mg/L, resp. Catechol, a fungal and bacterial metabolite of benzene, was found to be significantly more toxic to the biosensor than was the parent compd. benzene, showing that products of xenobiotic biodegrdn. may be more toxic than the parent compds. Combinations of parent compds. and metabolites were found to be significantly more toxic to the bioassay than were the individual compds. themselves. Development of this bioassay has provided a rapid screening system suitable for assessing the toxicity of xenobiotics commonly found in contaminated soil, river, and ground-water environments. The assay can be utilized over a wide pH range and is therefore more applicable to such environmental systems than bioluminescence-based bioassays that utilize marine organisms and can only be applied over a limited pH and salinity range.

Bury NR, McGeer JC, Eddy FB, Codd GA. **Liver damage in brown trout, Salmo trutta L., and rainbow trout, Oncorhynchus mykiss (Walbaum), following administration of the cyanobacterial hepatotoxin microcystin-LR via the dorsal aorta.** J Fish Dis 1997;20(3):209-15.
BIOSIS COPYRIGHT: BIOL ABS. Purified microcystin-LR (MC-LR) was administered via the dorsal aorta to brown trout, Salmo trutta L., or rainbow trout, Oncorhynchus mykiss (Walbaum), and, within 24

h, a dose of 300 mug MC-LR kg-1 caused increased activities in the blood by enzymes originating mainly from the liver, i.e. lactate dehydrogenase (LDH) and alanine transaminase (ALT). A dose of 75 mug MC-LR kg-1 significantly increased liver enzyme activities in the blood of brown trout at 24 h, but was without effect on rainbow trout, whereas 25 mug MC-LR kg-1 had no effect on blood LDH or ALT activities in either species. However, histopathological analysis of liver from both species following administration of the lowest toxin dose showed hepatocyte swelling and necrosis. Liver damage was more severe in brown trout compared to rainbow trout following administration of 300 mug MC-LR kg-1, showing disruption of the parenchymal architecture. After 48 h, there was a dose-dependent increase in the hepatosomatic index in both species. It is concluded that brown trout are less tolerant to MC-LR than rainbow trout.

De Bruijn JH., Hof M. **How to measure no effect: Part IV: how acceptable is the Ecx from an environmental policy point of view?** Environmetrics 1997;8(3):263-7.
BIOSIS COPYRIGHT: BIOL ABS. Recently, EC point estimation (ECx, a certain percentile of the concentration-effect curve) has been mentioned in scientific literature as an alternative to the concept of deriving a no observed effect concentration (NOEC) in toxicological and ecotoxicological tests. The question of whether the NOEC approach should be exchanged for the ECx approach concerns not only test guideline development, but has also consequences in the area of risk assessment and environmental quality objectives. Therefore, from an environmental policy point of view it is essential to look critically at the advantages and disadvantages of moving away from an NOEC to ECx estimation; in environmental policy the term 'no effect' as well as NOEC values themselves play an important role in legislation and procedures related to risk assessment and environmental quality objectives both at a national and an international level.

Ferrari L, Demichelis SO, Garcia ME, De La Torre FR, Salibian A. **Premetamorphic anuran tadpoles as test organism for an acute aquatic toxicity assay.** Environ Toxicol Water Qual 1997;12(2):117-21.
BIOSIS COPYRIGHT: BIOL ABS. An acute semistatic bioassay method for the evaluation of water quality in highly polluted river samples is described. Bufo arenarum young tadpoles as test organism were used. This anuran is a species of the native herpetofauna of Argentina. The technique was checked with surface water samples of Reconquista River, collected from four sites along the low-to-high pollution gradient. The controls were run with artificial hard water. The sensitivity of tadpoles to toxicants was checked by incubation of test animals in sublethal and lethal cadmium solutions as a standard toxicant. Mortality was registered daily for 96 h and the results were expressed as cumulative mortality. Statistical analysis was carried out by means of a multiway factor analysis of variance with Bonferroni range test. Each assayed sample was chemically analyzed in order to determine the content of heavy metals, and organochlorine and organophosphorous insecticides. This bioassay did allow us to discriminate clearly between high and low polluted samples showing a good toxicological correlation with the determined chemical profile.

Hoke RA, Ankley GT, Kosian PA, Cotter AM, Vandermeiden FM, Balcer M, Phipps GL, West C, Cox JS. **Equilibrium partitioning as the basis for an integrated laboratory and field assessment of the impacts of DDT, DDE and DDD in sediments.** Ecotoxicology 1997;6(2):101-25.
BIOSIS COPYRIGHT: BIOL ABS. Many of the most biologically productive portions of streams are

backwater areas which support large populations of benthic macroinvertebrates. The sediments in these locations and their associated macroinvertebrate communities are frequently subjected to chemical inputs and physical perturbations. Historically, assessment of the effects of contaminants in sediments have emphasized chemical analyses and either laboratory toxicity tests or in-stream monitoring of benthic macroinvertebrate community structure. However, combining the chemical and biological approaches provides a more powerful assessment technique. Such an integrated approach, combining laboratory water-only and sediment toxicity tests with Hyalella azteca and Chironomus tentans, field surveys of benthic macroinvertebrate community structure and evaluation of chemical data using equilibrium partitioning theory was used to assess the effects of DDT, DDE and DDD (collectively termed DDTR) in the sediments of the Huntsville Spring Branch-Indian Creek (HSB IC) stream system in the southeastern USA. Benthic macroinvertebrate populations in the HSB-IC system still appear to be adversely affected by DDTR residues within the sediments even though DDT discharges to the stream were stopped over 20 years ago and a major remediation project was completed in the late 1980s. This conclusion is based on a weight of evidence approach which incorporates (1) the observed sediment toxicity to C. tentans and H. azteca in laboratory tests, (2) the identification of DDTR as the likely cause of effects observed during laboratory toxicity.

Lipnick RL. **Environmental hazard assessment using lipophilicity data**. Methods Princ Med Chem 1996;4:339-53.

Prepas EE, Kotak BG, Campbell LM, Evans JC, Hrudey SE, Holmes CF. **Accumulation and elimination of cyanobacterial hepatotoxins by the freshwater clam Anodonta grandis simpsoniana.** Can J Fisheries Aquatic Sci 1997;54(1):41-6.
BIOSIS COPYRIGHT: BIOL ABS. Freshwater clams (Anodonta grandis simpsoniana) exposed to 51-55 mug L-1 of dissolved microcystin-LR (MC-LR) in the laboratory for 3 days did not accumulate MC-LR equivalents (MC-LReq). However, clams placed in three eutrophic lakes with phytoplankton containing MC-LR (concentrations from below detection to 8.3 mug L-1 cellular toxin) for 12-28 days accumulated the toxin (24 : 7 to 527 : 330 ng g-1 MC-LReq; mean : SE). The relative MC-LReq concentrations in clams reflected MC-LR concentrations in lake phytoplankton, but individual variation was high. In individual clams exposed for 24 days, the average MC-LReq concentration was usually greater in the visceral mass than in gills and muscle, but average toxin concentrations in the three tissues were similar (587, 310, and 364 ng g dry weight-1). In clams removed from the lake and placed in toxin-free water, MC-LReq concentrations in tissues declined rapidly for 6 days (by 69-88%) but remained relatively stable for the remaining 15 days. Analysis of clam tissues appears to be a more sensitive MC-LR indicator than analysis of phytoplankton. Accumulation of potent cyanobacterial toxins by this clam warrants further study as many are consumed by muskrats (Ondatra zibethicus), which in turn are consumed by terrestrial predators.

Rapala J, Sivonen K, Lyra C, Niemela SI. **Variation of microcystins, cyanobacterial hepatotoxins, in Anabaena spp. as a function of growth stimuli.** Appl Environ Microbiol 1997;63(6):2206-12.
Cyanobacterial hepatotoxins, microcystins, are specific inhibitors of serine/threonine protein phosphatases and potent tumor promoters. They have caused several poisonings of animals and also pose a health hazard for humans through the use of water for drinking and recreation. Different strains of

the same cyanobacterial species may variously be nontoxic, be neurotoxic, or produce several microcystin variants. It is poorly understood how the amount of toxins varies in a single strain. This laboratory study shows the importance of external growth stimuli in regulating the levels and relative proportions of different microcystin variants in two strains of filamentous, nitrogen-fixing Anabaena spp. The concentration of the toxins in the cells increased with phosphorus. High temperatures (25 to 30 degrees C), together with the highest levels of light studied (test range, 2 to 100 mumol m-2 s-1), decreased their amount. Different structural variants of microcystins responded differently to growth stimuli. Variants of microcystin (MCYST)-LR correlated with temperatures below 25 degrees C, and those of MCYST-RR correlated with higher temperatures. Nitrogen added into the growth medium and increasing temperatures increased the proportion of microcystin variants demethylated in amino acid 3. All variants remained mostly intracellular. Time was the most important factor causing the release of the toxins into the growth medium. Time, nitrogen added into the growth medium, and light fluxes above 25 mumol m-2 s-1 significantly increased the concentrations of the dissolved toxins. According to the results, it thus seems that the reduction of phosphorus loads in bodies of water might play a role in preventing the health hazards that toxic cyanobacterial water blooms pose, not only by decreasing the cyanobacteria but also by decreasing their toxin content.

Rojickova R, Marsalek B. **[Bacterial toxicity tests]**. Vodni Hospod 1997;47(5):158-60. (Cze)
CBAC COPYRIGHT: CHEM ABS A review and discussion with 11 refs. The objective of the paper is to inform the Czech experts in water management about the possibilities of application of several toxicity tests with bacteria that are com. available or simple to use. The following methodologies are described and discussed: Spirillum volutans motility inhibition, agar plate method, bacterial growth inhibition, ECHA Biocide Monitor, Toxi-Chromotest, Toxi-ChromoPad, MetPad, MetPlate, FluoroMetPAD, FluoroMetPLATE, MetSOIL, Polytox.

Russom CL, Bradbury SP, Broderius SJ, Hammermeister DE, Drummond RA. **Predicting modes of toxic action from chemical structure: acute toxicity in the fathead minnow (Pimephales promelas)'**. Environ Toxicol Chem 1997;16(5):948-67.
CBAC COPYRIGHT: CHEM ABS In the field of aquatic toxicol., quant. structure-activity relationships (QSARs) have developed as scientifically credible models for predicting the toxicity of chems. when little or no empirical data are available. In recent years, there has been an evolution of QSAR development and application from that of a chem.-class perspective to one that is more consistent with assumptions regarding modes of toxic action. The objective of this research was to develop procedures that relate modes of acute toxic action in the fathead minnow (Pimephales promelas) to chem. structures and properties. An empirically derived database for diverse chem. structures of acute toxicity and corresponding modes of toxic action was developed through joint toxic action studies, the establishment of toxicodynamic profiles, and behavioral and dose-response interpretation of 96-h LC50 tests. Using the results from these efforts, as well as principles in the toxicol. literature, approx. 600 chems. were classified as narcotics (three distinct groups), oxidative phosphorylation uncouplers, respiratory inhibitors, electrophiles/proelectrophiles, acetylcholinesterase inhibitors, or central nervous system seizure agents. Using this data set, a computer-based expert system has been established whereby chem. structures are assocd. with likely modes of toxic action and, when available, corresponding QSARs.

Schreiber S, Becker K, Bresler V, Fishelson L. **Dietary L-carnitine protects the gills and skin of guppies (Poecilia reticulata) against anionic xenobiotics**. Comp Biochem Physiol C 1997;117c(1):99-102.
CBAC COPYRIGHT: CHEM ABS L-Carnitine is a carrier of activated fatty acids into mitochondria, but it may also have other functions. Expts. were conducted to investigate possible influences of dietary L-carnitine at the cellular level. Contact fluorescent microscopy was used to compare the responses of tissues of fish fed different levels of dietary L-carnitine when exposed to the fluorescent markers fluorescein and acridine orange. The penetration and accumulation of these markers in living cells was estd. by measuring the intracellular intensity of their fluorescence (530 nm). The results showed that penetration of fluorescein from water via gills was significantly lower in L-carnitine fish than in control fish. Intact plasma membranes are almost impermeable to org. anions, such as fluorescein, but damage of plasma membranes increases their permeability. Thus, it appears that the membranes of L-carnitine fish may be better protected against the penetration of anionic xenobiotics than the membranes of control fish. Accumulation of acridine orange, a cationic compd., did not show any significant differences between L-carnitine fish and control fish. Org. cations penetrate plasma membranes via diffusion, and this is unlikely to be influenced by L-carnitine.

Sloterdijk H. **Estimation of no-effect concentrations of effluents based on log-logistically distributed NOEC toxicity data**. Can Tech Rep Fish Aquat Sci 1995;(2050):81-9.
CBAC COPYRIGHT: CHEM ABS A procedure for the estn. of a safe concn., using NOEC toxicity data, is presented. It is a statistical approach based on the theory that toxic responses (NOEC's, LC50's, etc.) are log-logistically distributed. A computer algorithm (called ETX) allows the extrapolation of percentiles with a certain confidence from a small set of toxicity data. The ETX algorithm, in essence, calcs. the 5th percentile of the hypothetical logistic distribution from which the data are thought to derive (lab. toxicity data). This 5th percentile can be considered a NOEC value, above which 95% of the species seem relatively safe. This algorithm was applied to toxicity data on effluents of 3 industries on the St. Lawrence River. The data include results from Microtox, algal, and Ceriodaphnia tests, and from the SOS-Chromotest (genotoxicity). The extrapolated NOEC values, expressed as toxic units (TU), can be used to rank the effluents, and to calc. mass loadings of TU's. Based on the effluent and the receiving water flowrates, diln. ratios can be calcd., which may indicate sufficient or insufficient mixing or diln.

Steinberg LJ, Reckhow KH, Wolpert RL. **Characterization of parameters in mechanistic models: a case study of a PCB fate and transport model.** Ecol Model 1997;97(1-2):35-46.
BIOSIS COPYRIGHT: BIOL ABS. As a first step in a Bayesian analysis of PCB fate and transport in the upper Hudson River, a joint probability density function for parameters in a simulation model is created. The density function describes the joint probabilities of the following parameters: the anaerobic dechlorination rate constant, the volatilization rate constant, the aerobic biodegradation rate constant, the sedimentation rate, and the contaminated sediment depth. Difficulties in forming this probability density function are shown to result from problems with extrapolating data from the laboratory to the field, non-stationarity and aggregation, extrapolating information and analyses from other sites, and bias due to study design. These difficulties result in a density function characterized by high variances, and imply that predictions from this simulation model, and similarly large fate-and-transport models, are apt to be highly uncertain. Bayesian analysis is proposed as a rigorous mathematical technique for including

observational data in density function generation in order to reduce prediction uncertainty.

Sun HW, Huang GL, Dai SG, Chen TY. **A diparametric QSAR pattern for organotin compounds on rotifer Brachionus plicatilis**. Toxicol Environ Chem 1997;60(1-4):75-85.

Versteeg DJ, Stanton DT, Pence MA, Cowan C. **Effects of surfactants on the rotifer, Brachionus calycifluorus, in a chronic toxicity test and in the development of Qsars.** Environ Toxicol Chem 1997;16(5):1051-8.
BIOSIS COPYRIGHT: BIOL ABS. The toxicity of a range of surfactant and surfactant-related compounds was investigated in the 2-d whole life cycle bioassay with the rotifer, Brachionus calyciflorus. Compounds were selected to gain an understanding of how structural components, especially the polar head groups, contribute to toxicity. Rotifers were exposed under static test conditions to the 22 compounds for 2d. Exposure concentrations were verified analytically in the test system. Results demonstrate a relationship between alkyl chain length and toxicity within a surfactant class. Between classes, N-containing amines and quaternary ammonium compounds had greatest toxicity, in general, followed by the nonionic compounds. Anionic compounds were typically least toxic. A good quality (R2 = 0.86), three-variable, parametric QSAR model was developed using the ADAPT software package. The model contains one variable to account for the contribution of the hydrophobic tail group to observed toxicity (the number of sp3-hybridized carbons bonded to two other carbons (2SP3)) and two descriptors, the valence-corrected, fourth-order cluster index (4XCv) and a count of the number of nitrogens in the molecule (NN), to account for the contribution of the polar head group.

GENOTOXICITY AND MUTAGENESIS

Adam W, Mielke K, Saha-Moller CR, Moller M, Stopper H, Hutterer R, Schneider FW, Ballmaier D, Epe B, Gasparro FF, et al. **Photochemical and photobiological studies of a furonaphthopyranone as a benzo-spaced psoralen analog in cell-free and cellular DNA.** Photochem Photobiol 1997;66(1):46-54.
Photobiological activities of the benzo-spaced psoralen analog furonaphthopyranone 3 have been investigated in cell-free and cellular DNA. The molecular geometry parameters of 3 suggest that it should not form interstrand crosslinks with DNA. With cell-free DNA no evidence for crosslinking but also not for monoadduct formation was obtained; rather, the unnatural furocoumarin 3 induces oxidative DNA modifications under near-UVA irradiation. The enzymatic assay of the photosensitized damage in cell-free PM2 DNA revealed the significant formation of lesions sensitive to formamidopyrimidine DNA glycosylase (Fpg protein). In the photooxidation of calf thymus DNA by the furonaphthopyranone 3, 0.29 +/- 0.02% 8-oxo-7,8-dihydroguanine (8-oxoGua) was observed. With 2'-deoxyguanosine (dGuo), the guanidine-releasing photooxidation products oxazolone and oxoimidazolidine were formed predominately, while 8-oxodGuo and 4-HO-8-oxodGuo were obtained in minor amounts. The lack of a significant D2O effect in the photooxidation of DNA and dGuo reveals that singlet oxygen (type II process) plays a minor role; control experiments with tert-butanol and mannitol confirm the absence of hydroxyl radicals as oxidizing species. The furonaphthopyranone 3 (Ered = -1.93 +/- 0.03V) should act in its singlet-excited state as electron acceptor for the photooxidation of dGuo (delta GET ca -6 kcal/mol), which corroborates photoinduced electron transfer (type I) as a major DNA-oxidizing mechanism.

A comet assay in Chinese hamster ovary (CHO) AS52 cells demonstrated that the psoralen analog 3 damages cellular DNA upon near-UVA irradiation; however, no photosensitized mutagenicity was observed in CHO AS52 cell cultures.

Adamson RH. **Sixth International Conference on carcinogenic-mutagenic n-substituted aryl compounds: conclusions and perspectives**. Mutat Res 1997;376(1-2):3-6.
BIOSIS COPYRIGHT: BIOL ABS. RRM JOURNAL ARTICLE HUMAN CARCINOGENESIS N-SUBSTITUTED ARYL COMPOUNDS 2-ACETYLAMINOFLUORENE NITROPYRENES NITRO-POLYCYCLIC AROMATIC HYDROCARBONS 4-AMINOBIPHENYL ISONIAZID HETEROCYCLIC AROMATIC AMINES MUTAGENICITY TOXICOLOGY EPIDEMIOLOGY.

Ali A, Krone PH, Pearson DS, Heikkila JJ. **Evaluation of stress-inducible hsp90 gene expression as a potential molecular biomarker in Xenopus laevis.** Cell Stress Chaperones 1996;1(1):62-9.
In this study we have evaluated stress-inducible hsp90 mRNA accumulation as a potential molecular biomarker in Xenopus laevis. In order to obtain a probe for Northern blot analysis we employed a PCR-based approach using degenerate primers for the amplification and cloning of an hsp90 gene sequence from Xenopus laevis. The deduced amino acid sequence is 102 amino acids in length and exhibited the highest degree of identity with zebrafish and human hsp90 beta genes. Furthermore, the putative intron and exon boundaries of this fragment are the same as hsp90 beta in chicken, mouse and human, indicating that the fragment represents a Xenopus hsp90 beta-like gene. Northern blot analyses revealed that this gene was constitutively expressed in cultured A6 cells. While heat shock and sodium arsenite exposure resulted in the increased accumulation of hsp90 mRNA in A6 cells, treatment with cadmium chloride and zinc chloride did not. Also, exposure of A6 cells to concurrent heat shock and sodium arsenite produced a mild synergistic response with respect to hsp90 mRNA levels in contrast to hsp70 mRNA levels which displayed a strong synergistic effect. Finally, hsp90 mRNA was detected constitutively throughout early embryogenesis but was heat-inducible only in late blastula and later stages of development. Given the normal abundance and limited stress-induced accumulation of hsp90 mRNA, it may not have a great deal of potential as a molecular biomarker compared to hsp70 and hsp30 mRNA. However, it may be useful in conjunction with other stress protein mRNAs to establish a set of biomarker profiles to characterize the cellular response to a stressful or toxic agent.

Anari MR, Josephy PD, Henry T, O'Brien PJ. **Hydrogen peroxide supports human and rat cytochrome P450 1A2-catalyzed 2-amino-3-methylimidazo[4,5-f]quinoline bioactivation to mutagenic metabolites: significance of cytochrome P450 peroxygenase.** Chem Res Toxicol 1997;10 (5):582-8.
We show that the naturally occurring hydroperoxide hydrogen peroxide is highly effective in supporting the cytochrome P450 1A2 peroxygenase-catalyzed metabolic activation of the heterocyclic aromatic amine 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) to genotoxic metabolites. Mutagenicity was assessed by the Ames assay with Salmonella typhimurium strain YG1012 and an activation system consisting of hydroperoxides plus either 3-methylcholanthrene-induced rat liver microsomes (rP4501A) or human P450 1A2-containing microsomes (hP4501A2). The mutagenic response was dependent on the concentration of microsomal protein, IQ, and hydroperoxides. The addition of hydrogen peroxide or tert-butyl hydroperoxide to rP4501A greatly enhanced the yield of histidine prototrophic (His+) revertants.

This increase was inhibited, in a concentration-dependent manner, by alpha-naphthoflavone, a P450 1A inhibitor. Hydrogen peroxide was the most effective peroxygenase cofactor, particularly with hP4501A2 (K(m) = 0.1 mM). The hydroperoxide-supported activation of IQ produced reactive intermediates which bound to 2'-deoxyguanosine; LC/MS analysis of the adducts revealed the same major (protonated) adduct at m/z = 464.4 as previously reported for the DNA adduct formed (in vivo or in vitro) by the mixed function-catalyzed bioactivation system. None of the peroxidase-catalyzed IQ metabolites (nitro-, azo-, or azoxy-IQ) were detected. In conclusion, hydrogen peroxide in the physiological/pathological concentration range may be able to support the metabolic activation of arylamines to genotoxic products through the cytochrome P450 peroxygenase pathway.

Anderson D, Dobrzynka MM, Jackson LI, Yu T, Brinkworth MH. **Somatic and germ cell effects in rats and mice after treatment with 1,3-butadiene and its metabolites, 1,2-epoxybutene and 1,2,3,4-diepoxybutane**. Mutat Res 1997;391(3):233-41.
CBAC COPYRIGHT: CHEM ABS The present study investigated somatic and germ cell effects of 1,3-butadiene in mice and its metabolites in both rats and mice to help det. species differences using different endpoints for genotoxic effects. These included DNA strand breakage as measured in the single cell gel electrophoresis (Comet assay) in bone marrow and testicular cells, and micronuclei in bone marrow cells using both the acridine orange and Giemsa staining methods. Unscheduled DNA synthesis (UDS) was also measured in the testes of mice. CD-1 mice were exposed to 1,3-butadiene by inhalation for 6 h/day for 4 wk, and CD-1 mice and Sprague-Dawley rats to the metabolites after i.p. injection. 1,3-Butadiene did not affect liver, bone marrow and testicular cells in mice as measured in the Comet assay. After treatment with 1,2-epoxybutene in the Comet assay, there was a response in the testes in mice but not in rats and there was little or no effect in the bone marrow assay in mice but there was in rats. After treatment with 1,2,3,4-diepoxybutane in the Comet assay in mice, there was a response in the bone marrow cells but not in the testicular cells, and in rats there was also a response only in bone marrow cells. There was an increase in micronuclei in both rats and mice with both metabolites, but clastogenicity was stronger with 1,2,3,4-diepoxybutane, occurring at lower doses, than with 1,2-epoxybutene. In the UDS assay in the testes of mice, there was an increase in response with 1,2,3,4-diepoxybutane treatment but not with 1,2-epoxybutene. These studies would appear to confirm a species difference of CD-1 mice and Sprague-Dawley rats, where mice were sensitive at lower doses than rats.

Anderson D, Yu TW, Browne MA. **The use of the same image analysis system to detect genetic damage in human lymphocytes treated with doxorubicin in the Comet and fluoresence in situ hybridisation (FISH) assays.** Mutat Res 1997;390(1-2):69-77.
Two assays, the alkaline single cell gel electrophoresis (Comet) assay and the fluorescence in situ hybridisation (FISH) of a whole chromosome or 'chromosome painting' assay have gained importance in recent years as witnessed by the increasing yield of scientific literature using these techniques. Thus, it would be useful to have one system to measure both endpoints. In the present communication, a cost-effective electronic imaging system developed by Kinetic Imaging Ltd., UK, has been used to measure, after treatment of human lymphocytes with doxorubicin, DNA damage in the Comet assay (using software package KOMET) and chromosome damage with the FISH technique (using software package KROMASCAN). The chromosome damage has been detected using chromoprobe-M for chromosome 1 and compared with chromosome damage measured using the conventional Giemsa staining technique. In

all three assays, cycling cells were treated, after phytohaemagglutinin stimulation, at 48 h for about 20 h, which resulted in statistically significant dose-related responses in each assay. In non-cycling cells there was no increase in damage in the Comet assay, but there was in the chromosome assays. The FISH assay was only conducted in cycling cells, since the probe used was metaphase-specific. At the highest doses of doxorubicin used, FISH and conventional chromosome assays had similar sensitivities.

Blagoeva PM, Mircheva TJ, Atanassova RB, Atanassov BT. **Genotoxic changes in the pulmonary alveolar macrophages of mice, rats and hamsters treated with tobacco smoke.** J Cancer Res Clin Oncol 1997;123(5):253-8.
To determine whether tobacco smoke (TS) is genotoxic for lung tissue macrophages (pulmonary alveolar macrophages, PAM) as a general result of its inhalatory action BD6 rats, Syrian golden hamsters and BDF1 (C57BlxDBA2) mice were subjected to wholebody exposure for 90 or 60 min daily (600 cm3 mainstream smoke in 16-1 glass chamber, 9 or 6 exposures of 15 min each, respectively), for different periods ranging up to 30 days. A significant enhancement of the frequency of polynucleated macrophages (BiN PAM) was observed in all animal species after more than 10-days of repeated exposure to TS. The increased level of BiN PAM (the number of bi- (+) poly-nucleated PAM) correlates with the duration of exposure to TS: on day 20 after the start of inhalation, more than 25/1000 of mice PAM were polynucleated.

Bubak A, Mielzynska D, Siwinska E. **Can we detect mutagenic activity of urinary sediment by the Ames test?** Int J Occup Med Environ Health 1997;10(1):47-54.
The urine of five children and four coke-oven workers in a Polish town was tested for mutagenic substances. Mutagenic activity was found in samples from both groups, but only in acetone extracts of the urinary filtrate and not in the urinary sediment.

Cammas FM, Pullinger GD, Barker S, Clark AJ. **The mouse adrenocorticotropin receptor gene: cloning and characterization of its promoter and evidence for a role for the orphan nuclear receptor steroidogenic factor 1**. Mol Endocrinol 1997;11(7): 867-76.
CBAC COPYRIGHT: CHEM ABS To elucidate the mechanism underlying the tissue-specific expression of the ACTH receptor/MC2 receptor (ACTH-R) in the adrenal cortex, the mouse ACTH-R promoter was cloned. Anal. of the cDNA 5'-end showed an untranslated region of 321 bp, and the ACTH-R gene was demonstrated to be composed of two exons of 113 and 112 bp lying upstream of the single coding exon. S1 nuclease protection assay showed 2 major transcription start sites sepd. by 4 bp; 1.8 kb of the 5'-flanking region inserted in a luciferase reporter vector had cell-specific promoter activity because it was functioned only in mouse adrenocortical Y1 cells but not in mouse Leydig TM3 cells or fibroblast L cells. There was no dramatic change in the promoter activity in Y1 cells for all the deletions tested up to -113 bp upstream of the transcription start site. In contrast, expression in TM3 cells was switched on from deletion -908 bp, while remaining undetectable in L cells. Site-directed mutagenesis of a steroidogenic factor 1 (SF1)-like site at position -25 bp resulted in a significant redn. in luciferase expression by the promoter in Y1 cells. Gel shift anal. of this site indicated specific binding of a protein in exts. of Y1 and TM3 cells. Moreover, expression of SF1 in L cells induced promoter activity of the construct p(908). In conclusion, cell-specific expression of the mouse ACTH-R appears to be controlled by .gtoreq.2 factors. One of them, most probably SF1, is responsible for steroidogenic cell-specific

expression. The other as yet unknown factor binding between position -1236 bp and -908 bp acts as a strong inhibitory factor in nonadrenal steroidogenic cells, resulting in the adrenal-specific expression of ACTH-R.

Cerda H, Delincee H, Haine H, Rupp H. **The DNA 'comet assay' as a rapid screening technique to control irradiated food.** Mutat Res 1997;375(2):167-81.
The exposure of food to ionizing radiation is being progressively used in many countries to inactivate food pathogens, to eradicate pests, and to extend shelf-life, thereby contributing to a safer and more plentiful food supply. To ensure free consumer choice, irradiated food will be labelled as such, and to enforce labelling, analytical methods to detect the irradiation treatment in the food product itself are desirable. In particular, there is a need for simple and rapid screening methods for the control of irradiated food. The DNA comet assay offers great potential as a rapid tool to detect whether a wide variety of foodstuffs have been radiation processed. In order to simplify the test, the agarose single-layer set-up has been chosen, using a neutral protocol. Interlaboratory blind trials have been successfully carried out with a number of food products, both of animal and plant origin. This paper presents an overview of the hitherto obtained results and in addition the results of an intercomparison test with seeds, dried fruits and spices are described. In this intercomparison, an identification rate of 95% was achieved. Thus, using this novel technique, an effective screening of radiation-induced DNA fragmentation is obtained. Since other food treatments also may cause DNA fragmentation, samples with fragmented DNA suspected to have been irradiated should be analyzed by other validated methods for irradiated food, if such treatments which damage DNA cannot be excluded.

Cheng R, Ford BL, O'Neal PE, Mathews CZ, Bradford CS, Thongtan T, Barnes DW, Hendricks JD, Bailey GS. **Zebrafish (Danio rerio) p53 tumor suppressor gene: cDNA sequence and expression during embryogenesis**. Mol Mar Biol Biotechnol 1997;6(2):88-97.
CBAC COPYRIGHT: CHEM ABS Three methods were used in succession to screen a whole adult zebrafish cDNA library for expressed p53-like genes. The sequences of the resultant clones describe an open reading frame 1122 nucleotides in length, with another 43 and 940 bases of 5' and 3' untranslated sequence, resp. The deduced amino acid sequence of the zebrafish p53 protein is 63% identical to that of trout and 48% identical to that of human p53. Two of the three zebrafish clones overlap to span the entire reported cDNA sequence and are identical in their deduced amino acid sequence over their coincident length. The third clone contains a conservative amino acid change, as well as an inserted amino acid subsequently found to be at the junction of exons 2 and 3, suggestive of alternative splicing in the p53 mRNA for this species. Northern anal. demonstrated a zebrafish p53-related transcript to be present and most abundant in zygotes and early-cleavage embryos less than 1 h after fertilization, thereafter declining to barley detectable levels at 48 h. A similar temporal expression was detected for the zebrafish L-myc, known to be present in maternally derived RNA, whereas zebrafish N-myc and the zebrafish homolog of the murine T gene were not detectable prior to the onset of zygotic transcription.

Clements C, Ralph S, Petras M. **Genotoxicity of select herbicides in Rana catesbeiana tadpoles using the alkaline single-cell gel DNA electrophoresis (comet) assay**. Environ Mol Mutagen 1997;29(3):277-88.
CBAC COPYRIGHT: CHEM ABS The authors examd. the DNA damage caused by five herbicides

commonly used in southern Ontario (Canada). Erythrocytes from Rana catesbeiana (bullfrog) tadpoles were evaluated for DNA damage following exposure to selected herbicides, using the alk. single-cell gel DNA electrophoresis (SCG) or comet assay. This approach involves detection, under alk. conditions, of DNA fragments that upon electrophoresis migrate from the nuclear core, resulting in a comet formation. The herbicides tested, along with their active ingredients, were AAtrex Nine-O (atrazine), Dual-96OE (metalochlor), Roundup (glyphosate), Sencor-500F (metribuzin), and Amsol (2,4-D amine). Tadpoles were exposed in the lab. for a 24-h period to several concns. of the herbicides dissolved in dechlorinated water. Me methanesulfonate was used as a pos. control. The herbicides AAtrex Nine-O-, Dual-96OE-, Roundup-, and Sencor-500F-treated tadpoles showed significant DNA damage when compared with unexposed control animals, whereas, Amsol-treated tadpoles did not. Unlike the other responding herbicides, Sencor-500F did not show a relation between dosage and DNA damage. In summary, the results indicate that at least some of the herbicides currently used in southern Ontario are capable of inducing DNA damage in tadpoles.

Collins AR, Dobson VL, Dusinska M, Kennedy G, Stetina R. **The comet assay: what can it really tell us?** Mutat Res 1997;375(2):83-93.

Corley-Smith GE, Lim CJ, Kalmar GB, Brandhorst BP. **Efficient detection of DNA polymorphisms by fluorescent RAPD analysis**. Biotechniques 1997;22(4):690, 692, 694, 696, 698-9.
CBAC COPYRIGHT: CHEM ABS A method is presented for the anal. of fluorescently labeled random amplified polymorphic DNA (FRAPD) fragments. A DNA sequencer and collection and anal. software were used to est. the sizes of DNA fragments based on their mobilities relative to in-lane size markers. This allowed confident identification and comparison of FRAPD markers both within and between polyacrylamide gels. In comparison with anal. of RAPD products using ethidium bromide-stained agarose gels, fluorescent anal. improved the sensitivity, resoln. and precision of sizing of RAPD products of about 50-2100 bp. FRAPD fragments produced from amplification of zebrafish DNA are informative as genetic markers that segregate with Mandelian inheritance. FRAPD anal. was found to be very efficient for identifying new DNA polymorphisms.

Daza P, Schubler H, McMillan TJ, Girod SC, Pfeiffer P. **Radiosensitivity and double-strand break rejoining in tumorigenic and non-tumorigenic human epithelial cell lines.** Int J Radiat Biol 1997;72 (1):91-100.
Radiosensitivity and repair of DNA damage induced by ionizing radiation and restriction enzymes were investigated in three human epithelial cell lines: two tumorigenic squamous carcinoma cell lines (SCC-4 and SCC-25), and a non-tumorigenic epidermal keratinocyte cell line (RHEK-1). Sensitivity to ionizing radiation was determined using a clonogenic cell survival assay, which showed SCC-4 to be more radiosensitive than SCC-25 and RHEK-1, which in turn displayed about equal sensitivity. Using DNA precipitation under alkaline conditions for the analysis of induction and repair of DNA single-strand breaks (ssb), an increased level of ssb induction was found for SCC-4 while the efficiency of ssb repair was about equal in all three cell lines. Using pulsed-field gel electrophoresis (PFGE) for the measurement of induction and repair of DNA double-strand breaks (dsb), no consistent differences were detected between the three cell lines. A plasmid reconstitution assay was used to determine the capacity to rejoin restriction enzyme-induced dsb in whole-cell.

De Boeck M, Kirsch-Volders M. **Nereis virens (Annelida: Polychaeta) is not an adequate sentinel species to assess the genotoxic risk (comet assay) of PAH exposure to the environment.** Environ Mol Mutagen 1997;30(1):82-90.
Polychaetes, because of their bioturbation capacity, play an important role in the distribution of anthropogenic contaminants (including polycyclic aromatic hydrocarbons [PAHs]) throughout the sediments. In this work the use of Nereis virens (Annelida: Polychaeta) as a bioindicator to assess the genotoxic risk of PAH exposure for the environment was evaluated. For this purpose the alkaline single cell gel electrophoresis [comet] assay was applied on the coelomocytes of in vivo exposed Nereis virens. Benzo[a]pyrene (B[a]P) was chosen because it is classified by the IARC (International Agency for Research on Cancer) as probably carcinogenic to humans and because its mechanisms of action are well-known. Nereis virens was exposed to B[a]P in concentrations of 0.3, 0.6, 10, 20, 35 and 45 mg/ml by an intracoelomic injection of B[a]P (20 microliters) dissolved in dimethyl sulphoxide (DMSO). A solvent control with DMSO, a positive control with ethyl methane sulphonate (EMS) (12.1 mg/ml) and a negative control were included in each experiment. For each treatment four animals were analysed. After 1 hr treatment coelomocytes were harvested by puncturing the coelomic cavity with a sharpened Pasteur pipette, mixed with 0.5% low melting point agarose and sandwiched between two other gel layers. Ethidium bromide stained nuclei were analysed for tail length and tail moment. 12.1 mg/ml EMS, pure DMSO (98.9%) and B[a]P in all tested concentrations induced a statistically significant increase of DNA single strand breaks in the comet assay. The effect of B[a]P, however, was only at the highest concentration (45 mg/ml) significantly stronger than the effect of DMSO alone. Although a relatively large heterogeneity in the results could be observed, these experiments clearly showed that Nereis virens is not suited as a sentinel species for the assessment of the genotoxic risk of PAH exposure because this species seems to be very resistant to benzo[a]pyrene.

Delaney CA, Green IC, Lowe JE, Cunningham JM, Butler AR, Renton L, D'Costa I, Green MH. **Use of the comet assay to investigate possible interactions of nitric oxide and reactive oxygen species in the induction of DNA damage and inhibition of function in an insulin-secreting cell line**. Mutat Res 1997;375(2):137-46.
CBAC COPYRIGHT: CHEM ABS In the present study, we have compared 3-morpholinosydnonimine (SIN-1), which generates nitric oxide, superoxide anion and hydrogen peroxide, with two other nitric oxide donors, S-nitrosoglutathione (GSNO) and the tetra-iron-sulfur cluster nitrosyl, Roussin's black salt (RBS). We have used the comet assay as a highly sensitive method to measure DNA-damaging ability, and also measured inhibition of DNA synthesis and inhibition of insulin secretion. We have examd. the effect of superoxide dismutase (SOD) and catalase on each of these endpoints in HIT-T15 cells following a 30-min exposure to the compds. (24 h for DNA synthesis). All compds. produced a significant dose-dependent increase in strand-breakage formation and all inhibited DNA synthesis and glucose-stimulated insulin secretion. RBS was the most potent. SOD did not reduce the responses obsd. with any of the compds. Catalase largely prevented DNA strand breakage, inhibition of DNA synthesis and inhibition of insulin secretion by SIN-1, but had no effect on responses to GSNO or RBS. Addn. of SOD together with catalase gave no greater protection against SIN-1 than catalase alone. The nitric oxide and superoxide anion produced by SIN-1 are thought to combine to form highly reactive peroxynitrite. In addn., H2O2 may be formed in the presence of SIN-1 and may form hydroxyl radical in

the presence of a transition metal, such as Fe2+. It appears that in insulin-secreting cells, the effects of SIN-1 are largely mediated by this latter mechanism. In contrast, GSNO and RBS appear to act by a different mechanism, not overtly involving reactive oxygen species. GSNO and H2O2 show no significant interaction in the induction of DNA strand breaks. Both nitric oxide and H2O2 are effective, directly or indirectly, as DNA strand-breaking agents, inhibitors of DNA synthesis and inhibitors of insulin secretion.

Delaney CA, Pavlovic D, Hoorens A, Pipeleers DG, Eizirik DL. **Cytokines induce deoxyribonucleic acid strand breaks and apoptosis in human pancreatic islet cells**. Endocrinology 1997;138(6):2610-4.
CBAC COPYRIGHT: CHEM ABS The authors have previously obsd. that a 6-day exposure of human pancreatic islets to a combination of cytokines (interleukin-1beta 50 U/mL + tumor necrosis factor-alpha 1000 U/mL + interferon-gamma 1000 U/mL) severely impairs beta-cell functions. In the present study, the authors examd. whether this condition affects DNA integrity and viability of human islet cells. Cells were studied after 3, 6, and 9 days of cytokine treatment by both single cell gel electrophoresis (the comet assay, a sensitive method for detection of DNA strand breaks) and by a cytotoxicity assay using the DNA binding dyes Hoechst 33342 and propidium iodide as indexes for the no. of viable, necrotic, and apoptotic cells. Cytokine treatment for 6 and 9 days resulted in a 50% increase in comet length (vs. controls), indicating DNA strand breaks, as well as in a significant increase in the no. of apoptotic cells (vs. controls), but not in the no. of necrotic cells. The arginine analogs NG-nitro-L-arginine and NG-monomethyl-L-arginine prevented nitric oxide formation by the cytokines but did not interfere with cytokine-induced DNA strand breaks and apoptosis. The present data suggest that prolonged (6-9 days) exposure of human pancreatic islets to a mixt. of cytokines induces DNA strand breaks and cell death by apoptosis. These deleterious effects of cytokines appear to be independent of nitric oxide generation.

Devaux A, Pesonen M, Monod G. **Alkaline comet assay in rainbow trout hepatocytes**. Toxicol In Vitro 1997;11(1-2): 71-9.
CBAC COPYRIGHT: CHEM ABS The alk. comet assay was performed to measure DNA integrity in fish hepatocytes. Primary cultures of rainbow trout hepatocytes were exposed to two known genotoxic compds., hydrogen peroxide (H2O2) and benzo[a]pyrene (B[a]P), and to org. exts. of river sediments. The DNA damage in the form of single-strand breaks was monitored following the formation of DNA comets after alk. electrophoresis. Exposure of the hepatocytes to H2O2 for 2 h increased strand breaks in a dose-related manner at the concn. range reported previously in studies with mammalian hepatocytes. B [a]P treatment led to a significant increase in strand breaks at the concns. ranging from 0.1 to 10 muM after 4 h of exposure. After 48 h of exposure to B[a]P, the level of DNA strand breaks was lower than that of the control. The org. exts. obtained from river sediments significantly increased DNA strand breaks in trout hepatocytes, indicating the presence of genotoxic compds. in the sediment. The results show that the alk. comet assay is a promising method by which to study the genotoxic potential of xenobiotics found in the aquatic environment.

Duan Y, Guttman SI, Oris JT. **Genetic differentiation among laboratory populations of Hyalella azteca: implications for toxicology**. Environ Toxicol Chem 1997;16(4):691-5.
BIOSIS COPYRIGHT: BIOL ABS. Hyalella azteca of different origins are maintained in many

laboratories and are extensively used in toxicological bioassays. Quality assurance of interlaboratory toxicity data can only be made when the amount of genetic differentiation among laboratory populations is known. We assayed genetic variability at 16 enzymatic loci in H. azteca stocks from six laboratories (2 U.S. Environmental Protection Agency (USEPA), 1 U.S. Fish and Wildlife Service (USFWS), University of Mississippi, Environment Canada, Burlington, ON, Canada, and New York Department of Environmental Conservation), as well as an additional species, Hyalella montezuma (used as an outgroup). Three divergent groups were identified in laboratory stocks of H. azteca based on both Nei's genetic identity and modified Rogers' genetic distance. These three groups were differentiated genetically at a level characteristic of distinct species (Nei's I = 0.26-0.69) based on the comparison between H. azteca and H. montezuma. The high level of genetic differentiation among populations indicated that the Burlington and New York populations were distinct species of Hyalella. In order to enhance interlaboratory toxicity test comparisons, we recommend using genetically characterized populations of H. azteca.

Elias Z, Daniere MC, Marande AM, Point O, Terzetti F, Schneider O. **Genotoxic and/or epigenetic effects of some glycol ethers: results of different short-term tests**. Occup Hyg 1996;2(1-6):187-212.
The purpose of this report was to contribute to a general view on the cytotoxic potential of some glycol ethers. Short term tests were performed to evaluate genotoxic and/or epigenetic effects of several glycol ethers. The studies included gene mutation at the HPRT locus in the V79 cells; sister chromatid exchanges in the V79 cells, and chromosomal aberrations in the V79 cells and human lymphocytes; micronuclei in polychromatic erythrocytes of mouse bone marrow and in-vitro in V79 cells; changes of the mitotic division apparatus and aneuploidy in V79 cells; morphological transformation of Syrian-hamster embryo cells; and inhibition of intercellular communication between V79 cells. The findings indicate that in in-vitro cell systems, the alkoxyacetaldehydes are the active metabolites of the ethylene-glycol ethers, expressing a genotoxic activity and an aneugenic potential in a dose dependent manner at non cytotoxic, low concentrations. The authors suggest that it is likely that a continuously dividing cell population is more exposed to the toxicity of these intermediate metabolites than a growth arrested cell population. In in-vitro short term tests, only the intrinsic potential of the alkoxyacetaldehydes to induce genotoxic and epigenetic effects was detected. They suggest that in-vivo studies will help determine whether or not the corresponding ethylene glycol ethers express this potential by their active metabolites.

Forster W, Scheid W, Weber J, Schurenberg M, Traut H, Busse H. **Fluence and mutagenic side effects of excimer laser radiation applied in ophthalmology in human lymphocytes in vitro.** Acta Ophthalmol Scand 1997;75(2):124-7.
PURPOSE: To investigate the influence of different fluences in 193 and 248 nm excimer laser radiation on the yields of chromatid and chromosome aberrations induced in human lymphocytes in vitro. METHOD: Heparinized human blood was exposed to 193 or 248 nm excimer laser radiation. The fluence was gradually increased from 21 to 400 mJ/cm2 in 193 nm (constant total energy of 250 J) and from 150 to 377 mJ/cm2 in 248 nm radiation (constant total energy of 500 J). Chromatid and chromosome aberrations were then analysed microscopically. RESULTS: The yields of chromatid breaks and achromatic lesions depend on the fluence per pulse. This dependence contains a linear component, indicating a threshold of about 70 mJ/cm2 fluence in 193 nm and of about 250 mJ/cm2

fluence in 248 nm laser radiation. An increase of the yield of dicentric chromosomes could only be observed at the highest fluence tested (377 mJ/cm2) in the 248 nm series. Over 126 mJ/cm2 in 193 nm radiation no lymphocytes could be cultured and therefore no aberrations could be found. CONCLUSIONS: Our findings show that the fluence of 193 nm and of 248 nm excimer laser radiation has an effect on the yields of chromatid breaks and achromatic lesions in human lymphocytes under in vitro conditions.

Glatt H. **Bioactivation of mutagens via sulfation.** FASEB J 1997;11(5):314-21.
BIOSIS COPYRIGHT: BIOL ABS. Sulfation is a common final step in the biotransformation of xenobiotics and is traditionally associated with inactivation. However, the sulfate group is electron-withdrawing and may be cleaved off heterolytically in some molecules, leading to an electrophilic cation. The stable heterologous expression of sulfotransferases in indicator cells of standard mutagenicity tests has substantially improved the accessibility of this activation pathway. Sulfotransferase-mediated genotoxic effects have been demonstrated for numerous benzylic alcohols derived from polycyclic aromatic hydrocarbons and various aromatic hydroxylamines. Also, hycanthone (a benzylic alcohol), alpha-hydroxytamoxifen (an allelic alcohol), 1'-hydroxysafrole (an allelic/benzylic alcohol), and 2-nitropropane are activated to genotoxicants by sulfotransferases. Various reactive sulfate conjugates show strong mutagenic effects only when they are generated directly within the indicator cell, due to their inefficient penetration of cell membranes. In other cases, secondary membrane-penetrating reactive species are formed from sulfuric acid esters by displacement reactions with medium components, such as chloride or amino acids. Reaction with water regenerates the alcohol, which becomes available for a new cycle of activation. Different sulfotransferases from the same species as well as related forms from rat and human differ in their substrate specificities and tissue distributions. These characteristics and reactivities of the sulfate conjugates formed may explain organotropic effects of the compounds activated via sulfotransferases.

Glatt H. **Sulfation and sulfotransferases 4: bioactivation of mutagens via sulfation.** FASEB J 1997;11(5):314-21.
Sulfation is a common final step in the biotransformation of xenobiotics and is traditionally associated with inactivation. However, the sulfate group is electron-withdrawing and may be cleaved off heterolytically in some molecules, leading to an electrophilic cation. The stable heterologous expression of sulfotransferases in indicator cells of standard mutagenicity tests has substantially improved the accessibility of this activation pathway. Sulfotransferase-mediated genotoxic effects have been demonstrated for numerous benzylic alcohols derived from polycyclic aromatic hydrocarbons and various aromatic hydroxylamines. Also, hycanthone (a benzylic alcohol), alpha-hydroxytamoxifen (an allylic alcohol), 1'-hydroxysafrole (an allylic/benzylic alcohol), and 2-nitropropane are activated to genotoxicants by sulfotransferases. Various reactive sulfate conjugates show strong mutagenic effects only when they are generated directly within the indicator cell, due to their inefficient penetration of cell membranes. In other cases, secondary membrane-penetrating reactive species are formed from sulfuric acid esters by displacement reactions with medium components, such as chloride or amino acids. Reaction with water regenerates the alcohol, which becomes available for a new cycle of activation. Different sulfotransferases from the same species as well as related forms from rat and human differ in their substrate specificities and tissue distributions. These characteristics and reactivities of the sulfate

conjugates formed may explain organotropic effects of the compounds activated via sulfotransferases.

Gornung E, Gabrielli I, Cataudella S, Sola L. **CMA3-banding pattern and fluorescence in situ hybridization with 18S rRNA genes in zebrafish chromosomes**. Chromosome Res 1997;5(1):40-6.
CBAC COPYRIGHT: CHEM ABS This study provides new data of zebrafish chromosomes, obtained from the chromomycin A3-banding pattern and mapping of 18S rRNA genes by fluorescence in situ hybridization (FISH). C-banding and Ag staining were also performed to analyze whether variation in heterochromatin and Ag-nucleolus organizer regions (NORs) exists among various com. purchased strains. The results provide information on heterochromatin compn. and on the existence in interindividual NOR polymorphism and contribute to the construction of an idiogram suitable for gene mapping.

Graser RT, Malnar-Dragojevic D, Vincek V. **Cloning and characterization of a 70 kd heat shock cognate (hsc70) gene from the zebrafish (Danio rerio)**. Genetica 1997;98(3):273-6.
CBAC COPYRIGHT: CHEM ABS The heat shock 70 family of proteins is one of the most highly conserved among all species. The genes encoding these proteins have been cloned and sequenced from bacterial species to humans with a high degree of homol. preserved throughout evolution. Here the authors describe the cloning and characterization of a cDNA encoding a 70 kd heat shock cognate (hsc70) gene from the zebrafish (Danio rerio). A high degree of conservation is obsd. among hsc70 genes of other species as shown by phylogenetic anal. The characterization of a hsc70 gene in the zebrafish provides a marker for studying the role of a constitutively expressed member of the hsp70 family in an important developmental and evolutionary model system.

Graves RJ, Green T. **Mouse liver glutathione S-transferase mediated metabolism of methylene chloride to a mutagen in the CHO/HPRT assay**. Mutat Res 1996;367(3):143-50.
The relationship between methylene-chloride (75092) (MC) induced DNA single strand breaks and hypoxanthine-ribosyltransferase (HPRT) mutation, and the nature of MC/glutathione (GSH) compounds involved in DNA damage, were investigated. CHO-K1 cells were exposed to 5 millimolar (mM) GSH and 0.5% (volume/volume) MC. MC was weakly mutagenic and occurred in the absence of cytotoxicity. The mutagenicity was enhanced five to seven fold by exposing the cells in suspension. In two of three experiments, mutations were induced at single optimum mouse liver S100 cytosol fraction concentration of 20%. Mutation frequency increased in a dose dependent manner from 20 to 25% S100 and was sustained at 30% S100 without toxicity. MC was required to cause mutations at 30% S100. The mutagenic potential of MC was reduced by GSH depleted S100 fraction. MC in the presence of mouse liver S100 fraction caused an increase in DNA single strand breaks in cells exposed in suspension, but only marginal increases in DNA protein crosslinking. Slight increases in DNA single strand breaks were also seen with MC alone and S100 alone, although this effect was not seen when cells were exposed to higher concentrations of MC as attached cultures, and there was no evidence of mutagenicity. The authors conclude that MC is metabolized to a mutagen by mouse liver cytosol in a reaction that requires GSH, and DNA single strand breaks appear related to DNA mutations rather than cytogenetic damage.

Gunzel P, Reimann R. **Mutagenic carcinogens and noncarcinogens in transgenic mice [letter]**. Environ Health Perspect 1997;105(2):163.

Harvey JS, Parry JM. **The detection of genotoxin-induced DNA adducts in the common mussel Mytilus edulis.** Mutagenesis 1997;12(3):153-8.
In order to establish the capacity of Mytilus spp. to form genotoxin-DNA adducts, a series of in vitro and in vivo studies were conducted in which tissue samples and animals were exposed to five model genotoxins. Following the in vitro characterization of the major adducts induced by the compounds, a series of in vivo studies were conducted to determine if the levels of genotoxin-DNA adduct formation followed a dose response. The results of these studies suggested that under appropriate conditions, DNA adducts in the hepatopancreas could be used as molecular dosimeters of exposure to genotoxic compounds in the species. However, these studies also revealed that the successful detection of such genotoxin-DNA adducts depends largely upon their chromatographic properties and thus the vigour of the characterization undertaken.

Hatch FT, Colvin ME. **Quantitative structure-activity (QSAR) relationships of mutagenic aromatic and heterocyclic amines.** Mutat Res 1997;376(1-2):87-96.
We extended our previous studies of mutagenic/carcinogenic heterocyclic aromatic amines formed during the cooking of foods to 66 aromatic and 99 heterocyclic amines for which mutagenic potency data are available. The amines require activation by enzymes to form metabolites reactive with DNA and exhibit an enormous range of potency as frameshift mutagens in the Ames/Salmonella assay. To ascertain factors that might influence potency, structural features and quantum mechanical parameters calculated by the Huckel method (and, for a subset of 20 amines, by semi-empirical AM1, and ab initio methods) were analyzed by multiple linear regression. The major findings were: (1) earlier findings on cooked food mutagens and their synthetic congeners can be extended to other amines; (2) mutagenic potency is directly related to the number of fused aromatic rings (size of the aromatic system), the number of ring nitrogen atoms (participation of lone electron pairs in the pi-cloud), and presence of a methyl substituent on a ring nitrogen; (3) potency is inversely related to the energy of the lowest unoccupied molecular orbital (LUMO) of the parent amine. Ford and Griffin (1992) and Sabbioni and Wild (1992) showed that the LUMO energy of the derived nitrenium ion is closely related to its stability (calculated with reference to aniline). Increased stability has been hypothesized to enhance the probability of adduct formation with DNA by avoiding detoxifying side reactions and increasing the lifetime of the ion. In the large heterogeneous series of amines in our present study the Huckel method energy of the highest occupied molecular orbital (HOMO), rather than the LUMO energy, of the nitrenium ion was marginally related to the potency of the parent amine. However, in the selected subset of 20 amines with ab initio calculation, the LUMO energy of the ion confirmed the previous reports. The contribution of quantum chemical factors to mechanistic insight on the mutagenicity and carcinogenicity of aromatic and heterocyclic amines is still under development.

Helbig R, Speit G. **DNA effects in repair-deficient V79 Chinese hamster cells studied with the comet assay.** Mutat Res 1997;377(2):279-86.
Using the alkaline comet assay (single cell gel electrophoresis), we studied the induction and persistence of DNA damage induced by methyl methanesulfonate (MMS) and neocarzinostatin (NCS) in the repair-deficient Chinese hamster cell lines V-E5 and XR-V15B. Effects in the comet assay were analyzed directly after treatment as well as after a postincubation period in mutagen-free medium to gain insight

into the DNA repair capacities of the mutant cell lines in relation to different primary DNA lesions. Both mutagens caused a concentration-related increase in DNA strand breakage in both mutant cell lines and in the normal parental cell lines. Repair of MMS-induced DNA damage during postincubation was similar in normal and mutant cell lines, while diminished repair was seen after NCS treatment in XR-V15B cells. Our data show that XR-V15B cells only repaired about 30% of NCS-induced DNA damage within 1 h, while the parental V79 cell line repaired about 70%. Since this cell line is defective in the repair of DNA double-strand breaks (DSB), the results indicate that NCS-induced DSB significantly contribute to the genotoxic effects seen in the comet assay. However, compared to previously studied induction of gene mutations and chromosome aberrations, detection of NCS-induced DNA effects with the comet assay was less sensitive and increased DNA migration only occurred under strong cytotoxic conditions.

Inoue K, Yamazaki H, Imiya K, Akasaka S, Guengerich FP, Shimada T. **Relationship between CYP2C9 and 2C19 genotypes and tolbutamide methyl hydroxylation and S-mephenytoin 4'-hydroxylation activities in livers of Japanese and Caucasian populations.** Pharmacogenetics 1997;7 (2):103-13.
Genomic DNA was isolated from livers of 39 Japanese and 45 Caucasians and the genotypes of CYP2C9 and 2C19 genes were determined with PCR methods using synthetic oligonucleotide primers. Liver microsomes were also prepared from these human samples and activities for tolbutamide methyl hydroxylation and S-mephenytoin 4'-hydroxylation were determined. The single base mutation of C416T (Arg144Cys) in CYP2C9 was detected in 22% of Caucasians but not in Japanese samples. Another single base mutation at A1061C (Ile359Leu) in the CYP2C9 gene was found with frequencies of about 8% in both races. We did not detect any individuals who have either homozygous Cys144/ Cys144 or Leu359/Leu359 CYP2C9 variant nor both heterozygous Cys144-Ile359 and Arg144-Leu359 CYP2C9 variant in the human samples examined. The CYP2C19m2 genetic polymorphism was found only in Japanese people, while CYP2C19m1 type was determined in both races, with higher incidence in Japanese than in Caucasian population. Immunoblotting analysis of human liver microsomes suggested that CYP2C9 is a major component of the human CYP2C enzyme pool; it accounted for approximately 20% of total P450 in liver microsomes of both human populations. The levels of CYP2C19 protein were determined to be about 0.8% and 1.4% of total P450 (mean) in Japanese and Caucasians, respectively. We did not detect CYP2C19 protein in liver microsomes of humans who were genotyped for CYP2C19 gene as m1/m1, m1/m2, and m2/m2 variants but detected CYP2C9 protein in all of the samples examined. Good correlations were found between levels of CYP2C9 and activities of tolbutamide methyl hydroxylation (r = 0.77) and between levels of CYP2C19 and activities of S-mephenytoin 4'-hydroxylation (r = 0.86) in liver microsomes of the human samples examined. Tolbutamide methyl hydroxylation activities were lower in human samples with the Leu359 allele of CYP2C9 than those with the Cys144 allele and wild-type (Arg144-Ile359); the former type showed slightly higher K(m) values. When calculated on P450 basis, liver microsomes of individuals having m1/m1, m1/m2, and m2/ m2 types of CYP2C19 had very low catalytic activities for S-mephenytoin 4'-hydroxylation. These results provide useful comparisons for pharmacokinetic and toxicokinetic models of some of the clinically used drugs that are oxidized by CYP2C proteins in humans.

Kharchevnikova NV, Zholdakova ZI, Zhurkov VS, Poliakova EE, Novikov SM. **[Structure-mutagenic**

activity relationships on the TA100 strain of Salmonella typhimurium exposed to a series of halogenated hydrocarbons and short-chain alcohols]. Genetika 1997;33(5):710-3. (Rus)

Kinashi Y, Masunaga S, Takagaki M, Ono K. **Mutagenic effects at HPRT locus induced in Chinese hamster ovary cells by thermal neutrons with or without boron compound.** Mutat Res 1997;377 (2):211-5.
CHO cells were exposed to thermal neutrons and their mutation frequency was determined. The Kyoto University Research Reactor (KUR), which has a very low level of contamination by gamma-rays and fast neutrons was used as a thermal neutron source. Cells were irradiated in the presence or absence of boric acid to determine mutation frequency and cell survival. Thermal neutron irradiation was 2.5 times as mutagenic as gamma-irradiation without boron. In the presence of boron, however, thermal neutron irradiation was from 4.2 to 4.5 times as mutagenic as gamma-irradiation. When the mutation frequency was plotted against the survival fraction, a higher degree of mutagenicity was observed in the presence than in the absence of boron. These results suggest that the enhancement of thermal neutron-induced mutation with boron is strongly associated with alpha-particles released by 10B(n, alpha)7 Li reaction.

Kolman A, Spivak I, Naslund M, Dusinska M, Cedervall B. **Propylene oxide and epichlorohydrin induce DNA strand breaks in human diploid fibroblasts.** Environ Mol Mutagen 1997;30(1):40-6.
The induction of DNA strand breaks in human diploid fibroblasts (VH-10) was demonstrated after in vitro exposure with two carcinogenic epoxides, propylene oxide (PO) and epichlorohydrin (ECH). Alkaline DNA unwinding (ADU), pulsed field gel electropharosis (PFGE), and the comet assay were used to measure DNA single. (SSBs) and double-strand breaks (DSBs). A dose-dependent increase of DNA strand breaks, measured by ADU, was observed in the dose range 2.5-20 mMh of PO and 0.25-2 mMh of ECH. The dose-response of ECH was about five times higher compared with that of PO (211 vs. 41 SSBs. 100 Mbp-1.mMh-1). The induction rates of DSBs, measured by PFGE, were found to be 18 times higher for ECH compared to PO (4.8 and 0.27 DSBs.100 Mbp-1.mMh-1 for ECH and PO, respectively). Using these two methods, the SSBs/ DSBs ratio was estimated to be 148 for PO and 44 for ECH. The data obtained by the comet assay also demonstrated a dose-dependent ability of PO and ECH to induce DNA damage. It was found that ECH was about six times more effective as an inducer of DNA strand breaks compared to PO (200 and 32x100 Mbp-1.mMh-1 for ECH and PO, respectively). The SSBs/DSBs ratios calculated using comet assay and PFGE data were 125 for ECH and 41 for PO. In addition, ECH is about 10 times more toxic than PO with respect to survival. These properties of ECH can at least in part be explained by its higher chemical reactivity connected with a higher rate of DNA alkylation.

Latinwo LM, Ikediobi CO, Singh NP, Sponholtz G, Fasanya C, Riley L. **Comparative studies of in vivo genotoxic effects of cadmium chloride in rat brain, kidney and liver cells.** Cell Mol Biol (Noisy Le Grand) 1997;43(2):203-10.
BIOSIS COPYRIGHT: BIOL ABS. Cadmium chloride-induced DNA damage was investigated in individual brain, kidney and liver cells isolated from rats gavaged 14 mg/kg/day cadmium chloride. Animals were sacrificed on days 2, 4, 8, 16, and 33, and DNA damage was determined using the recently developed alkaline microgel electrophoresis technique. Data for DNA migration from 50 randomly selected cells clearly show significant increases in DNA damage in cells from three different

organs of cadmium chloride gavaged animals compared to saline treated control animals (33 day control, brain 64.7 : 5.3, kidney 75.5 : 9.4, liver 67.9 : 5.7 gm; 33 days experimental, brain 284.3 : 16.9, kidney 397.9 : 11.3, liver 315 : 22.5 gm; these values represent length of exposure in days and length of DNA migration in micron). There was an increase in DNA damage for all three cell types, with increasing duration of treatment. Cadmium (CdCl2) induced levels of DNA single strand breaks were more pronounced in kidney cells than in cells from the other two organs. Body and organ weights decreased of treated animals were decreased as compared to control. Results of this study indicate a potential of cadmium to be a genotoxic compound.

Lebailly P, Vigreux C, Godard T, Sichel F, Bar E, Letalaer JY, Henry-Amar M, Gauduchon P. **Assessment of DNA damage induced in vitro by etoposide and two fungicides (carbendazim and chlorothalonil) in human lymphocytes with the comet assay.** Mutat Res 1997;375(2):205-17.
BIOSIS COPYRIGHT: BIOL ABS. The effects of two fungicides (carbendazim and chlorothalonil) on the induction of DNA damage in human peripheral blood lymphocytes (human PBL) have been investigated using the single cell gel electrophoresis assay (SCGE assay or comet assay) immediately after a 1-h treatment and after a 24-h post-treatment incubation. The assessment of etoposide (an effective antitumour agent) effects on human PBL in terms of cell viability and dose-DNA damage relationships was made and etoposide selected as a positive control. The results indicate that etoposide induces significant ($p < 0.01$) dose-dependent DNA damages for concentrations at which the loss of cell viability is low. After a 24-h recuperation period, all observed DNA damages had disappeared. With SCGE assay performed after a 1-h treatment, similar positive results were observed with chlorothalonil alone or in association with carbendazim, without any loss of cell viability. However, a dramatic loss of cell viability was measured after 24 h and was associated with a large proportion of highly damaged cells. In contrast, carbendazim was not cytotoxic on human PBL and did not induced DNA damage using the SCGE assay either immediately after treatment or after a 24-h post-treatment incubation. These results point to the necessity of an adequate evaluation of immediate and long-term cytotoxicity of compounds that are to be assessed by the SCGE assay.

Liu X, Han S, Baluda MA, Park NH. **HPV-16 oncogenes E6 and E7 are mutagenic in normal human oral keratinocytes.** Oncogene 1997;14(19):2347-53.
The mutation frequency of pS189 shuttle vector plasmids is higher in human oral keratinocytes (NHOK) immortalized with cloned human papillomavirus-16 (HPV-16) genome than in primary normal NHOK (NHOK). To determine whether oncoproteins E6 and E7 of HPV-16 are responsible for the higher mutation frequency of the plasmids, we measured the mutation frequency in NHOK and in NHOK expressing the HPV-16 oncogenes (E6, E7, or E6 plus E7). We also measured the mutation frequency in NHOK expressing the E6 or E7 proteins of the non-oncogenic HPV-6b. The mutation frequency, either background or N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced, in NHOK expressing the HPV-16 oncoproteins (E6, E7, or E6 plus E7) was significantly higher than in NHOK. The HPV-16 oncogenes did not alter the nature of the MNNG-induced mutations (G:C-->A:T), but increased the frequency of deletions and insertions with or without MNNG. The background or MNNG-induced mutation frequency in NHOK expressing the HPV-6b E6 or E7 proteins was the same as in NHOK. NHOK and NHOK expressing HPV6b-E6 or E7 were able to arrest the cell cycle and enhance cellular p53, p21(WAF1/CIP1), and Gadd45 levels when exposed to MNNG, whereas NHOK expressing the

HPV-16 E6 oncogene did not demonstrate. NHOK expressing HPV-16 E7 were able to enhance cellular p53, p21(WAF1/CIP1), and Gadd45 levels, but failed to arrest cell cycle progression when exposed to MNNG. These data indicate that HPV-16 E6 and E7 oncogenes are mutagenic in human oral keratinocytes and enhance the mutagenic effect of MNNG. However, the E6 and E7 proteins of the 'low risk' HPV-6b did not demonstrate such an ability.

Malashenko AM, Beskova TB, Vasil'eva SV. **[Mutagenic effect of nitrosodimethylurea in male mice. Induction of dominant lethal mutations and chromosome aberrations in germ cells; genotype influence on the clastogenic effect in bone marrow cells]**. Genetika 1997; 33(4):524-31. (Rus)
The ability of dimethylnitrosourea (DMNU) to induce dominant lethal mutations in germ cells and chromosome aberrations in bone marrow cells of male CBAB6F1 mice was studied. At a mutagen dose of 200 mg/kg, mortality was 19% in the second week after treatment (being 5% in the control) and the frequency of bone marrow cells containing chromosome aberrations was 19.6% 24 h after treatment. DMNU induces dominant lethals in postmeiotic cells and in spermatogonia; the effects in spermatozoids and spermatogonia are equal. Chromosome aberrations in spermatocytes induced by DMNU are not realized as dominant lethals. The sensitivity of mouse strains to the clastogenic effect of DMNU ranged in an order similar to that observed in experiments with thioTEPA. The most sensitive was the TPS strain (29.2 +/- 4.6% of cells with chromosome aberrations), the most resistant-the CBA/Lac strain (9.5 +/- 2.9%). DMNU exhibited a relatively poor clastogenic activity; the effect in bone marrow cells was higher than that in male germ cells.

Marczewska J, Koziorowska J. **Comparison of the induction of SOS repair in Escherichia coli PQ37 and PQ243 by antineoplastic agents**. Acta Pol Pharm 1997;54(1):35-41.
CBAC COPYRIGHT: CHEM ABS Six alkylating antineoplastic drugs (Cyclophosphamide, Chlorambucil, Busulfan, Melphalan, Streptozotocin and Lomustine) and two ref. compds. (Me methanesulfonate and N-methyl-N'-nitro-N-nitrosoguanidine) were investigated in the SOS Chromotest using the Escherichia coli strain PQ37 (wild-type) and derived strain (PQ243), which carries the same markers as PQ37 and addnl. tagA alkA. As a measure of the SOS induced activity, induction factors of sfiA::lacZ expression were detd. Strain PQ243 was more sensitive towards all compds. in inducing SOS DNA repair than strain PQ37. Cyclophosphamide was detected as neg. in strain PQ243 in the presence of an exogenous metabolic activation system. Lomustine was inactive both in the mutant strain and in the wild-type strain in the presence of an S9 mix fraction as well as in the absence of it. Melphalan and Busulfan (without or with S9 mix) were shown to be pos. exclusively in strain PQ243. The authors we discuss the usefulness of strain PQ243 in monitoring of the genotoxicity of drugs and in the genetic anal. of their mode of action.

Matsuda T, Yagi T, Kawanishi M, Matsui S, Takebe H. **Molecular analysis of mutations induced by 2-chloroacetaldehyde, the ultimate carcinogenic form of vinyl chloride, in human cells using shuttle vectors**. Carcinogenesis 1995:16(10):2389-94.
The mutagenic effects of 2-chloroacetaldehyde (107200) (CAA), a metabolite of vinyl-chloride (75014) (VC), was studied in-vitro using molecular biology techniques. A modified shuttle vector plasmid pMY189 was constructed, then treated with 0.13 to 0.51 molar (M) CAA for 1 hour. Following treatment, the plasmids were transfected into a human fibroblast cell line and recovered. Plasmids

containing supF mutations were detected using an indicator Escherichia-coli strain. DNA sequencing was performed to determine the base sequences of the supF gene. CAA treatment increased supF mutation frequency in repair proficient and repair deficient cells. Approximately seven and 40 fold increases in mutation frequency over background were seen with 0.13 and 0.51M CAA, respectively. Base sequence analysis revealed that up to 90% of the single base substitutions were guanine/cytosine (G/C) base pair substitutions, with 54% of these being G/C to adenine/thymidine (A/T) transitions. Eight sites had four or more single base substitutions. Most of the mutations involving G/C base pairs occurred in the 5'-AAGG-3' or 5'-CCTT-3' sequences, indicating that these areas might be the major targets of CAA induced mutagenesis.

McCarthy PJ, Sweetman SF, McKenna PG, McKelvey-Martin VJ. **Evaluation of manual and image analysis quantification of DNA damage in the alkaline comet assay**. Mutagenesis 1997;12(4):209-14.

McGregor D. **A review of some properties of ethylene glycol ethers relevant to their carcinogenic evaluation**. Occup Hygiene 1996;2(1-6):213-35.
The results of mutagenicity and related tests with ethylene glycol ethers (EGEs) and their metabolites are summarized and reviewed, and properties of EGEs relevant to carcinogenicity are discussed. Ethylene-glycol ethers and their alkyloxyacid metabolites were found to be generally inactive in the genetic tests. The alkyloxyaldehyde metabolites, however, have shown positive results. Methoxyacetaldehyde (10312831) (MALD) has induced mutations in the Salmonella-typhimurium assay in TA-97a. Additionally, MALD, butoxyacetaldehyde (29043898) (BALD), and ethoxyacetaldehyde (22056822) (EALD) have induced sister chromatid exchanges, chromosomal aberrations, aneuploidy and micronuclei in mammalian cells in-vitro. MALD, however, did not induce micronuclei in bone marrow cells of mice injected with the compound (BALD and EALD were not tested). Inhibition of gap junctional intercellular communication was not observed in V79 cells exposed to any of the three alkyloxyacetaldehydes, and transformation of SHE cells was noted only on exposure to MALD. The author indicates that these test can either give information about possible mechanisms by which known carcinogens might act or they can be used as predictive assays which indicate a certain probability that an agent may be carcinogenic. No carcinogenicity test results have been published for EGEs. The use of mutagenicity tests to predict the carcinogenicity of EGEs is considered.

McGregor WG, Wei D, Chen RH, Maher VM, McCormick JJ. **Relationship between adduct formation, rates of excision repair and the cytotoxic and mutagenic effects of structurally-related polycyclic aromatic carcinogens.** Mutat Res 1997;376(1-2):143-52.

McKee RH, Vergnes JS, Galvin JB, Douglas JF, Kneiss JJ, Andrews LS. **Assessment of the in vivo mutagenic potential of methyl tertiary-butyl ether.** J Appl Toxico 1997l;17(Suppl 1):31-6.
Methyl tertiary-butyl ether (MTBE) is one of the highest production volume chemicals in the USA. Previous results from in vitro genetic toxicity studies suggested that it was not mutagenic. However, chronic exposure at high levels resulted in liver tumors in female mice and kidney tumors in male rats. The current program assessed in vivo genotoxicity and also explored the possibility that a mutagenic mechanism was involved in the carcinogenic process. The specific tests used included the Drosophila sex-linked-recessive-lethal test, the rat bone marrow cytogenetics test, the mouse bone marrow

micronucleus test and the in vivo-in vitro hepatocyte unscheduled DNA synthesis test in the mouse. All tests produced negative results, indicating that the potential for in vivo mutagenic activity was low. These data also suggest that the tumorigenic activity was probably the result of a non-genotoxic process.

McNair FI, Marples B, West CM, Moore JV. **A comet assay of DNA damage and repair in K562 cells after photodynamic therapy using haematoporphyrin derivative, methylene blue and meso-tetrahydroxyphenylchlorin.** Br J Cancer 1997;75(12):1721-9.
Single-cell electrophoresis (comet assay) has been used to evaluate DNA damage and repair in the human myeloid leukaemia cell line K562 after low-dose (predominantly sub-lethal) treatments of hyperthermia and photodynamic therapy (PDT). Three different photosensitizers were examined: haematoporphyrin derivative (HpD), methylene blue (MB) and meso-tetrahydroxyphenylchlorin (mTHPC). None of the drugs in the absence of light, nor in light alone, resulted in detectable DNA damage. However, a significant amount of DNA damage was detected immediately after treatment with haematoporphyrin derivative or methylene blue PDT compared with drug-only or light-only treatments; no residual level of DNA damage was evident for either drug following a 4-h post-treatment incubation at 37 degrees C. No significant DNA damage was detected after meso-tetrahydroxyphenylchlorin PDT or hyperthermia either immediately or 4 h after treatment. We conclude that the alkaline comet assay can be applied as an effective screening assay for DNA damage induced by a range of laser therapies.

Moore MM, Harrington-Brock K, Doerr CL. **Relative genotoxic potency of arsenic and its methylated metabolites.** Mutat Res 1997; 386(3):279-90.
BIOSIS COPYRIGHT: BIOL ABS. Arsenic is one of the few identified human carcinogens that has yet to be shown to cause cancer in rodents when the standard bioassay protocols are used. The reasons for this apparent interspecies difference are unclear but may be related to differences between humans and rodents in their detoxification capabilities. Detoxification of arsenic may occur through a methylation pathway. If, in fact, methylation does detoxify arsenic, one would predict that the methylated arsenicals might be less genotoxic than the inorganic arsenicals. To evaluate the hypothesis that the inorganic arsenicals are more mutagenic than the organic arsenicals, we tested sodium arsenite, sodium arsenate, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) for their relative mutagenic and clastogenic potentials. We used the L5178Y/TK+/- mouse lymphoma assay which allows the detection of chemicals inducing a broad spectrum of different types of genetic damage. Sodium arsenite and sodium arsenate were active at concentrations of 1-2 mug/ml and 10-14 mug/ml, respectively. MMA was active between 2500-5000 mug/ml; while DMA required almost 10000 mug/ml to induce a genotoxic response. The organic arsenicals are thus much less potent as mutagenic agents than the inorganic arsenicals. All four of these arsenicals appear to act by mechanisms that cause chromosomal mutations.

Mozzherin DJ, Shibutani S, Tan CK, Downey KM, Fisher PA. **Proliferating cell nuclear antigen promotes DNA synthesis past template lesions by mammalian DNA polymerase delta.** Proc Natl Acad Sci U S A 1997;94(12):6126-31.
Consistent with previous observations, proliferating cell nuclear antigen (PCNA) promotes DNA synthesis by calf thymus DNA polymerase delta (pol delta) past several chemically defined template lesions including model abasic sites, 8-oxo-deoxyguanosine (dG) and aminofluorene-dG (but not

acetylaminofluorene-dG). This synthesis is potentially mutagenic. The model abasic site was studied most extensively. When all deoxyribonucleoside triphosphates and a template bearing a model abasic site were present, DNA synthesis by pol delta beyond this site was stimulated 53-fold by addition of homologous PCNA. On an unmodified template (lacking any lesions), PCNA stimulated pol delta by 1.3-fold. Product analysis demonstrated that as expected from the A-rule, fully and near-fully extended primers incorporated predominantly dAMP opposite the template lesion. Moreover, corollary primer extension studies demonstrated that in the presence (but not the absence) of PCNA, pol delta preferentially elongated primers containing dAMP opposite the model abasic template site. p21, a specific inhibitor of PCNA-dependent DNA replication, inhibits PCNA-stimulated synthesis past model abasic template sites. We propose that DNA synthesis past template lesions by pol delta promoted by PCNA results from the fundamental mechanism by which PCNA stimulates pol delta, i.e., stabilization of the pol delta. template-primer complex.

Napolitano RL, Fuchs RP. **New strategy for the construction of single-stranded plasmids with single mutagenic lesions.** Chem Res Toxicol1997;10(6):667-71.
Single-stranded DNA vectors containing single adducts offer a unique opportunity to study the biochemistry and genetics of trans lesion synthesis, a process during which a DNA polymerase synthesizes across a lesion. We describe a new and general strategy to produce high-quality single-stranded plasmids containing a single adduct within a predetermined sequence context starting with a short oligonucleotide containing the lesion of interest. These vectors are isolated from the corresponding double-stranded constructs by selective enzymatic degradation in vitro of the nonadducted uracil-containing strand. Efficient and complete removal of this strand was achieved using uracil DNA glycosilase to generate AP sites followed by the action of the AP endonuclease associated with exonuclease III and the robust 3'-->5' exonuclease activity associated with T7 DNA polymerase. We show the utility of these constructs for the study of trans lesion synthesis in vitro and in vivo in the case of the highly carcinogenic N-2-acetylaminofluorene adducts located within frameshift mutation hot spots. The possibility to construct both single-stranded and double-stranded plasmids, with the same origin of replication (i.e., ColE1), will allow a direct comparison between single-stranded and double-stranded DNA replication in site-specific mutagenesis studies.

Parton JW, Garriott ML. **An evaluation of micronucleus induction in bone marrow and in hepatocytes isolated from collagenase perfused liver or from formalin-fixed liver using four-week-old rats treated with known clastogens.** Environ Mol Mutagen 1997;29(4):379-85.
The bone marrow (BM) micronucleus (MN) test is a sensitive assay for identifying clastogens. However, some clastogenic compounds and metabolites may never reach the BM. The liver has been suggested as an alternative tissue to BM but adult rat liver has a low mitotic index that increases the difficulty of evaluating hepatocytes (HEP) for MN induction. Chemical mitogens and partial hepatectomy have been used to increase HEP proliferation to improve the sensitivity for detection of clastogenic compounds, but these practices raise concerns for the evaluation of drug candidates. The use of 4-wk-old rats provides an alternative to mitogenic stimulation because livers from these animals have approximately 5.4% of their HEP in S-phase. HEP were isolated by collagenase perfusion, or from formalin-fixed tissue, from 4-wk-old treated rats. Six compounds were evaluated for the incidence of MN in HEP that were isolated by both methods. The results for MN induction by these compounds were

similar for the two methods and confirmed that formalin-fixed tissue is an acceptable source of cells for evaluating MN induction in HEP. BM polychromatic erythrocytes (PCE) also were harvested at the end of the live phase for each study and then evaluated for the incidence of MN. Diethylnitrosamine and 2-nitrofluorene induced MN in HEP but had no effect in PCE. 2-Acetylaminofluorene, cyclophosphamide and 7,12-dimethylbenz[a]anthracene did not induce MN in HEP but were positive in PCE. The direct-acting clastogen, mitomycin C, was positive in both HEP and PCE. These results indicate that this modified liver micronucleus test, using 4-wk-old rats, offers an alternative to existing methods that use mitogens or partial hepatectomy to stimulate cell replication. Analysis of MN from formalin-fixed tissue provides additional flexibility by allowing the investigator to assess MN induction at a later time.

Postlethwait JH, Talbot WS. **Zebrafish genomics: from mutants to genes**. Trends Genet 1997;13 (5):183-90.
CBAC COPYRIGHT: CHEM ABS A review with 63 refs. Exquisite embryonic lethal mutations have been isolated in hundreds of genes necessary for zebrafish development. Anal. of this resource promises to enhance our understanding of the mol. genetic mechanisms of vertebrate development. This review discusses the state of the zebrafish genome project and the genetic trickery that can expedite mol. isolation of genes disrupted by these mutations.

Ralph S, Petras M. **Genotoxicity monitoring of small bodies of water using two species of tadpoles and the alkaline single cell gel (comet) assay.** Environ Mol Mutagen 1997;29(4):418-30.
To monitor genotoxicity in small bodies of water (e.g., creeks, ponds, and drainage ditches) we examined tadpole erythrocytes of two species: Rana clamitans and Rana pipiens,using the alkaline single cell gel DNA electrophoresis (SCG) or comet assay. This approach involves detection, under alkaline conditions, of cell DNA fragments which on electrophoresis migrate from the nuclear core, resulting in a comet with tail formation. Fifty-six samples, a total of 606 tadpoles, from 18 sites in southern Ontario, collected between 1993 and 1995, were examined. Samples of R. clamitans tadpoles collected in 1994 and 1995, from regions with heavy agricultural activity, gave significantly higher ($P < 0.001$) DNA length to width ratios than samples of R. clamitans tadpoles collected from sites in the Bruce Peninsula and near the French River, which have little or no agriculture. Samples of R. pipiens tadpoles collected in 1994 from sites on the outskirts of Windsor, Ontario, sites which receive genotoxic inputs from nearby industries, gave significantly higher ($P < 0.001$) DNA ratios than samples from agricultural areas and the Bruce Peninsula. R. clamitans tadpoles showed significant annual variation in DNA damage which was greater in samples of tadpoles collected from agricultural areas than from the Bruce Peninsula. The higher levels of DNA damage in tadpoles collected from agricultural areas may be due to the pesticides used, and the increased variation in DNA damage in the same areas is likely due to the impact of crop rotation, including leaving fields fallow, the timing of rainfall, and/or the application of pesticides. R. clamitans tadpoles, especially those collected from agricultural areas, also showed significant seasonal variation in DNA damage. There was no significant ($P > 0.05$) seasonal or annual variation in the levels of DNA damage in R. pipiens tadpoles collected from the Tallgrass Prairie. This study indicates that both species are suitable for use in the alkaline SCG assay and as in situ sentinel organisms for environmental biomonitoring.

Re JL, De Meo MP, Laget M, Guiraud H, Castegnaro M, Vanelle P, Dumenil G. **Evaluation of the**

genotoxic activity of metronidazole and dimetridazole in human lymphocytes by the comet assay. Mutat Res 1997;375(2):47-55.
The genotoxicity of metronidazole (MZ) and dimetridazole (DZ) has been evaluated in human lymphocytes using the comet assay. The test has been performed using 3 doses (58.4, 175.2 and 292.1 microM for MZ; and 70.9, 212.6 and 354.3 microM for DZ) under 3 experimental protocols: aerobiosis, anaerobiosis (90% N2, 10% CO2) and with the presence of the microsomal fraction S9 mix. The effects of 4 antioxidants (8-hydroxyquinoline (8HQ), vitamin C (VitC), catalase (CAT) and superoxide dismutase (SOD), have been investigated on DNA damage generated by fixed concentrations of MZ (292.1 microM) and DZ (354.4 microM). In aerobic conditions, MZ and DZ produced significant dose-response relationships. The dose-related effects of both drugs decreased or were abolished in anaerobic conditions or in presence of S9 mix. 8HQ, VitC, CAT and SOD induced dose-related protective responses against DNA damage due to MZ and DZ. These findings suggest that MZ and DZ induce DNA damage in human lymphocytes through the futile cycle. The one-electron reduction of the drugs leads to the production of nitro radical anions. In the presence of oxygen, these radicals are reoxidized and generate oxygen-activated species.

Ruiz MJ, Marzin D. **Genotoxicity of six pesticides by Salmonella mutagenicity test and SOS chromotest.** Mutat Res 1997;390(3):245-55.
Two in vitro tests (Ames test and SOS chromotest), one for bacterial mutagenicity and one for primary DNA damage, were assayed to determine the genotoxic activity of 6 pesticides (atrazine, captafol, Captan, chlorpyrifosmethyl, molinate and tetrachlorvinphos). Assays were carried out both in the absence and presence of S9 fractions of liver homogenate from rat (Sprague-Dawley) pretreated with Aroclor 1254. Captan and captafol were genotoxic on both the Ames test and the SOS chromotest. Comparisons with mutagenesis data in Salmonella indicated that the SOS assay detected as genotoxic the pesticides that were mutagenic on the Salmonella test. Non-genotoxic effects were not detected in vitro either in the Salmonella/microsome assay nor in the SOS chromotest when bacterial tester strains were exposed to atrazine, molinate, chlorpyrifosmethyl and tetrachlorvinphos in the absence or presence of S9 mix.

Sasaki YF, Izumiyama F, Nishidate E, Matsusaka N, Tsuda S. **Detection of rodent liver carcinogen genotoxicity by the alkaline single-cell gel electrophoresis (Comet) assay in multiple mouse organs (liver, lung, spleen, kidney, and bone marrow)**. Mutat Res 1997;391(3):201-14.
CBAC COPYRIGHT: CHEM ABS We have recently designed a simple method for applying the alk. single-cell gel electrophoresis (SCG) assay to mouse organs. With this method, each organ is minced, suspended in chilled homogenizing buffer contg. NaCl and Na2EDTA, gently homogenized using a Potter-type homogenizer set in ice, and then centrifuged nuclei are used for the alk. SCG assay. In the present study, we used the method to assess the genotoxicity of 8 rodent hepatic carcinogens in 5 mouse organs (liver, lung, kidney, spleen, and bone marrow). The carcinogens we studied were p-aminoazobenzene, auramine, 2,4-diaminotoluene, p-dichlorobenzene, ethylene thiourea (ETU), styrene-7,8-oxide, phenobarbital sodium, and benzene-1,2,3,4,5,6-hexachloride (BHC); except for p-aminoazobenzene, they do not induce micronuclei in mouse bone marrow cells. Mice were sacrificed 3 and 24 h after the administration of each carcinogen. p-Aminoazobenzene, ETU, and styrene-7,8-oxide induced alk. labile DNA lesions in all of the organs studied. Auramine, 2,4-diaminotoluene, p-

dichlorobenzene, and phenobarbital sodium also produced lesions, but their effect was greatest in the liver. BHC, which is not genotoxic in in vitro tests, did not show any effects. We suggest that it may be possible to use the alk. SCG assay to detect in vivo activity of chems. whose genotoxicity is not expressed in bone marrow cells.

Sasaki YF, Nishidate E, Izumiyama F, Matsusaka N, Tsuda S. **Simple detection of chemical mutagens by the alkaline single-cell gel electrophoresis (Comet) assay in multiple mouse organs (liver, lung, spleen, kidney, and bone marrow)**. Mutat Res 1997;391(3):215-31.
CBAC COPYRIGHT: CHEM ABS We tested the genotoxicity in mouse organs of 11 chem. mutagens with different modes of action. Mice were sacrificed 3 and 24 h after administration of each mutagen. Treatment with three alkylating agents (MMS, EMS, and MNNG), a DNA crosslinking agent (MMC), two arom. amines (2-AAF and phenacetin), a polycyclic arom. hydrocarbon (B[a]P), and two inorg. chems. (KBrO3 and K2CrO4) increased migration of the DNA from mouse organs. 5-FU (a base analog) and colchicine (a spindle poison) treatment produced neg. results in all organ studied. Considering that the alk. SCG assay detects genotoxicity as DNA fragments derived from DNA single-strand breaks and alkali-labile damage, our results showed that the SCG assay using our homogenization technique detected chem. mutagens as a function of their modes of action.

Saxena S, Ashok BT, Musarrat J. **Mutagenic and genotoxic activities of four pesticides: captan, foltaf, phosphamidon and furadan.** Biochem Mol Biol Int 1997;41(6):1125-36.
The mutagenic and genotoxic potential of four pesticides viz. captan, foltaf, phosphamidon and furadan was evaluated by the Ames mutagenicity assay and their DNA damaging ability on radiation repair defective E. coli K-12 strains respectively. The mutagenic spectrum revealed captan to be most mutagenic in the absence of metabolic activation, while the presence of S9 mix led to an attenuated mutagenic response. Foltaf, phosphamidon and furadan were detected as relatively weaker mutagens. A significant decrease in the survival of SOS defective mutants, recA, lexA and pol- of E. coli was observed as compared to their wild-type counterparts in the presence of the pesticides. The role of SOS repair genes gains further support from the Salmonella strains triggering the error-prone SOS response.

Scarfi MR, Lioi MB, D'ambrosio G, Massa R, Zeni O, Di Pietro R, Di Berardino D. **Genotoxic effects of mitomycin-C and microwave radiation on bovine lymphocytes**. Electro Magnetobiol 1996;15 (2):99-107.
The possibility that microwave radiation may produce genotoxic effects as assessed by cytokinesis block micronucleus (MN) assay in bovine peripheral blood lymphocytes cultures was investigated. Blood was obtained from Italian Friesian-cattle, and Jersey-cattle and lymphocyte cultures were established. Cultures were exposed to microwave radiation at 9 gigahertz, with a specific absorption rate of 70 milliwatts, for 10 minutes. Mitomycin-C (MMC) was used as a positive control. Some cultures from microwave exposed samples were treated with MMC to evaluate possible cooperative effects. Four concentrations of MMC were tested, 0.022, 0.033, 0.044, and 0.055 micrograms/milliliter. The findings suggested that the optimal dose of MMC needed to induce MN frequency increases in this species was 0.044 micrograms/milliliter. The microwave radiation induced a statistically significant increase of MN frequency both with and without MMC, and a cooperative effect was seen. The authors suggest that aspects correlated with reproduction and low fertility should be considered.

Scassellati-Sforzolini G, Pasquini R, Moretti M, Villarini M, Fatigoni C, Dolara P, Monarca S, Caderni G, Kuchenmeister F, et al. **In vivo studies on genotoxicity of pure and commercial linuron**. Mutat Res 1997;390(3):207-21.
CBAC COPYRIGHT: CHEM ABS The ureic herbicide linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea] (CAS 330-55-2) was investigated for genotoxicity in a series of in vivo expts. Since human exposure to herbicides is not only to the active principles, but also to all the chems. present in the com. formulation, we tested both pure and com. linuron. Groups of rats were treated with gavage contg. different doses of the herbicide (pure compd. or com. formulation) for 14 days. The doses were 150, 300 and 450 mg/kg b.wt. for the pure compd. and 315.8, 631.6 and 947.4 mg/kg b.wt. for the com. formulation (47.5% of linuron). Feces and urine were collected at regular intervals. Urine specimens were analyzed for their mutagenic metabolites, thioethers and D-glucaric acid content. Feces exts. were tested for mutagenicity. Linuron's ability to cause DNA damage and cytogenetic effects was also investigated after treating groups of rats once with different doses of pure or com. linuron. DNA single-strand breaks were assessed in rat liver using the alk. elution technique and the single-cell microgel electrophoresis assay (SCGE: 'comet' assay), and in rat testes cells with the SCGE assay. Micronuclei induction was analyzed in rat bone marrow erythrocytes. Results obtained were mainly neg. when the excretion of mutagenic metabolites in urine and feces of animals treated with the pure compd. or with the linuron-based com. formulation were monitored, whereas an increase in the urinary excretion of thioethers and D-glucaric acid was obsd. in rats treated with the com. formulation. No increase in the frequency of micronucleated polychromatic erythrocytes was obsd. in the treated animals. However, linuron affected the viability of hepatocytes isolated from animals treated with higher doses. This cytotoxicity was accompanied by the induction of DNA single-strand breaks in the liver, as seen by the alk. elution assay. The potential of pure linuron to induce in vivo DNA damage was confirmed with the microgel electrophoresis technique ('comet' assay). Cytotoxicity was also seen in rat testes cells. However, no indication of DNA damage was visible.

Shimizu T, Mutoh H, Kato S. **Platelet-activating factor receptor: gene structure and tissue-specific regulation**. Adv Exp Med Biol 1996;416(2):79-84.
CBAC COPYRIGHT: CHEM ABS A review with several refs. The human platelet-activating factor receptor gene exists as a single copy on chromosome 1. Two 5'-noncoding exons (Exon 1 and 2) has distinct transcription initiation sites and promoters. These exons are alternatively spliced to a common splice acceptor site on exon 3 that contains a total coding regions. The transcript 1 is expressed ubiquitously with an emphasis of differentiated eosinophilic cell line (Eol-1), and leukocytes. On the other hand, the transcript 2 is expressed tissue-specifically. The latter is not expressed in leukocytes or brain. The transcript 1 has three tandem repeats of NF-kappaB, and SP-1 site, and responded to various inflammatory reagents including PAF itself, lipopolysaccharide, or phorbol ester. By northern blotting of tissue or cells with various nutritional or hormonal treatments, the PAF receptor messages are up-regulated. Estrogen increased the expression of the PAF receptor in human endometrial glandular cells, and vitamin A (retinoic acid) or thyroid hormone treatment up-regulates the PAF receptor expression only tissues with transcript 2. By various in vivo and in vitro transcriptional assays (CAT reporter assay, gel mobility shift assay), the authors identified estrogen responsible element, and hormone responsive element. The PAF receptor hormone responsive element is composed of three direct repeated TGACCT-

like hexamer motifs with 2 and 4 bp spaces, and the two upstream and two downstream motifs were identified as response elements for RA and T3.

Shmakova NL, Krasavin EA, Govorun RD, Fadeeva TA, Koshlan' IV. **[The lethal and mutagenic actions of radiations with different LETs on mammalian cells]**. Radiats Biol Radioecol 1997; 37 (2):213-9. (Rus)
Inactivation and induction of mutations in the HGPRT locus in Chinese hamster cells after irradiation with accelerated heavy ions in the LET range of 20 to 367 keV/micron were studied. In both cases, inactivation and induction of mutations, the LET dependence of RBE is described by a curve with a local maximum in the range of 80 to 100 keV/micron. The maximum RBE value for the mutagenic action is almost twice as high as that for inactivation. However, the RBE coefficients of the mutation induction criterion for a certain level of cell-survival is lower significantly and tend to decrease with an increase in inactivation. The obtained data show that the mutagenic effects, induction of chromosome aberrations, and deaths have their origins in the same kind of primary damages, i.e., double-strand breaks of DNA.

Siim BG, Menke DR, Dorie MJ, Brown JM. **Tirapazamine-induced cytotoxicity and DNA damage in transplanted tumors: relationship to tumor hypoxia**. Cancer Res 1997;57(14):2922-8.
CBAC COPYRIGHT: CHEM ABS This study examd. whether the enhancement of radiation damage to tumors by tirapazamine (TPZ) can be predicted from TPZ-induced DNA damage as measured by the comet assay. DNA damage provides a functional end point that is directly related to cell killing and should be dependent on both reductive enzyme activity and hypoxia. TPZ potentiated tumor cell kill by fractionated radiation in 3 murine tumors (SCCVII, RIF-1, and EMT6) and 2 human tumor xenografts (A549 and HT29), with no potentiation in a 3rd xenograft (HT1080). Overall, there was no correlation between radiation potentiation and TPZ-induced DNA damage in the tumors, except that the nonresponsive tumor xenograft had lower levels of DNA damage than the other 5 tumor types. However, there was a large tumor-to-tumor variability in DNA damage within each tumor type. This variability appeared not to result from differences in activity of the reductive enzymes but largely from differences in oxygenation between individual tumors, as measured by fluorescent detection of the hypoxia marker EF5. The results suggest that the sensitivity of individual tumors to TPZ, although not necessarily the response to TPZ plus radiation, might be assessed from measurements of DNA damage by the comet assay.

Tan J, Chan S. **Efficient gene transfer into zebrafish skeletal muscle by intramuscular injection of plasmid DNA**. Mol Mar Biol Biotechnol 1997;6(2):98-109.
CBAC COPYRIGHT: CHEM ABS The ability of zebrafish skeletal muscles to internalize and express plasmid DNA was demonstrated.

Tice RR, Nylander-French LA, French JE. **Absence of systemic in vivo genotoxicity after dermal exposure to ethyl acrylate and tripropylene glycol diacrylate in Tg.AC (v-Ha-ras) mice.** Environ Mol Mutagen 1997;29(3):240-9.

Tice RR, Yager JW, Andrews P, Crecelius E. **Effect of hepatic methyl donor status on urinary excretion and DNA damage in B6C3F1 mice treated with sodium arsenite**. Mutat Res 1997;386 (3):315-34.
CBAC COPYRIGHT: CHEM ABS This study evaluated the effect of hepatic Me donor status on the ability of sodium arsenite (2.5, 5.0 and 10.0 mg/kg) administered by gavage once or on four consecutive days to induce DNA damage in male B6C3F1 mice. Maintenance on a choline-deficient (CD) diet prior to treatment resulted in mice with hepatic Me donor deficiency (HMDD) and altered arsenical metab., as demonstrated by a decreased total urinary excretion of inorg. and org. arsenicals. The alk. (pH>13) Single Cell Gel (SCG) assay was used to evaluate for the induction of DNA damage (single strand breaks, alkali labile sites, DNA crosslinking) in blood leukocytes, liver parenchymal cells, and cells sampled from bladder, lung, and skin, while the bone marrow erythrocyte micronucleus (MN) assay was used to assess for the induction of chromosomal damage in bone marrow cells. Treatment with sodium arsenite once or four times induced a significant decrease in DNA migration (indicative of DNA crosslinking) in bladder and liver parenchymal cells of hepatic Me donor sufficient (HMDS) mice, but in skin cells of HMDD mice. Both HMDD and HMDS mice exhibited a significant increase in the frequency of micronucleated polychromatic erythrocytes (MN-PCE) in bone marrow following four, but not following one, treatments. However, the pos. response occurred at a lower dose for HMDS mice and, in these mice, bone marrow toxicity, as demonstrated by a significant redn. in the percentage of PCE, was present also. These results indicate that hepatic Me donors deficiency significantly decreases the total urinary excretion of orally administered sodium arsenite and markedly modulates target organ arsenic-induced DNA damage, with an apparent shift from liver and bladder to skin.

Varentsova ER, Khromykh IU. **[Interaction of mutations of the genes for mutagen sensitivity mei-9, mei-41 and rad201 as affected by ionizing radiation]**. Genetika 1997;33(3):328-32. (Rus)
Double mutants mei-41D5; rad(2)201G1 and mei-9a; rad(2)201G1 were constructed to study the interaction of these mutations in Drosophila exposed to gamma-rays. mei-9 and mei-41 mutants are sensitive to the lethal effects of a broad spectrum of chemical.

Wakabayashi K, Totsuka Y, Fukutome K, Oguri A, Ushiyama H, Sugimura T. **Human exposure to mutagenic/carcinogenic heterocyclic amines and comutagenic beta-carbolines.** Mutat Res 1997;376 (1-2): 253-9.
BIOSIS COPYRIGHT: BIOL ABS. Various kinds of mutagenic and carcinogenic heterocyclic amines (HCAs) are produced by heating protein-rich foods, such as meat and fish. To evaluate the risk of these HCAs in terms of human cancer development, exposure levels must be measured. We therefore analyzed their amounts in various kinds of cooked foods and in urine samples of healthy volunteers living in Tokyo. Based on the obtained quantitative data, daily exposure levels to 2-amino-3,8-dimethylimidazo(4,5f)quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP) were calculated to be 0.3-3.9 and 0.005-0.3 mug per person, respectively. Moreover, human DNA samples were analyzed with the 32P-postlabeling method, and colon, rectum and kidney tissues were found to contain an adduct spot corresponding to the standard 5'-pdG-C8-MeIQx by TLC and HPLC, at levels of 14, 18 and 1.8 per 1010 nucleotides, respectively. The beta-carboline compound, norharman, is produced by heating L-tryptophan, and is known to be present in cooked foods and in cigarette smoke at higher levels than mutagenic and carcinogenic HCAs. While norharman is not itself

mutagenic to Salmonella, it does become mutagenic to S. typhimurium TA98 with S9 mix in the presence of non-mutagenic aromatic amines like aniline and o-toluidine. When we examined whether DNA adducts are formed in the DNA of S. typhimurium TA98 by treatment with norharman and aromatic amines using 32P-postlabeling analysis, DNA adduct formation by norharman with aromatic amines was found to be related to the appearance of mutagenicity by norharman with aromatic amines.

Ward TH, Butler J, Shahbakhti H, Richards JT. **Comet assay studies on the activation of two diaziridinylbenzoquinones in K562 cells**. Biochem Pharmacol 1997;53(8):1115-21.

Watanabe-Akanuma M, Shimoi K, Kinae N, Ohta T. **Food-derived heterocyclic amines potentiate the mutagenicity of a drinking water mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX).** Mutat Res 1997;377(2):225-9.
We investigated the enhancing effect of heterocyclic amines on base-substitution mutations with 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1) and 2-amino-3,4-dimethyl-imidazo[4,5-f] quinoline (MeIQ). We compared the mutagenicity of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) in the presence and absence of the heterocyclic amines in E. coli WP2 (trpE) and in excision repair-deficient strains WP2s (uvrA, trpE) and ZA500 (uvrA, rfa, trpE). Since the assay was performed without microsomal metabolic activation, Trp-P-1 and MeIQ alone were not mutagenic. In WP2, trp+ reversions induced by MX were greatly potentiated by Trp-P-1 and slightly potentiated by MeIQ. Mutation enhancement was not observed in strains WP2s and ZA500, suggesting that a functional DNA excision repair system is necessary for the combined action of MX and heterocyclic amines. Our finding implies that the combined effect of mutagens as well as the effect of individual mutagens, should be considered in risk evaluation.

Willett CE, Cherry JJ, Steiner LA. **Characterization and expression of the recombination activating genes (rag1 and rag2) of zebrafish**. Immunogenetics 1997;45(6):394-404.

Wood DJ, Macadam AJ. **Laboratory tests for live attenuated poliovirus vaccines.** Biologicals 1997;25 (1):3-15.
A new generation of tests to control live attenuated poliovirus vaccines are under development based on major advances in our understanding of the molecular basis of attenuation and reversion to virulence of polioviruses. These include an alternative in vivo neurovirulence test in transgenic mice that express the human poliovirus receptor and a new in vitro test, the MAPREC (mutant analysis by polymerose chain reaction and restriction enzyme cleavage assay, that assesses consistency of production at a molecular level. Excellent progress is being made with both methods but neither is sufficiently developed yet for regulatory use. Critical review of existing control tests shows that the WHO neurovirulence test is well standardized and contributes significantly to the assessment of each batch. On the other hand, the current rct40 test is neither standardized nor particularly informative, though improvements could be made in both areas. The continued relevance of other marker tests such as the d or antigenic marker is doubtful. Potency, identity and thermal stability tests are crucial for control of the final trivalent vaccine.

Woods JA, Young AJ, Gilmore IT, Morris A, Bilton RF. **Measurement of menadione-mediated DNA**

damage in human lymphocytes using the comet assay. Free Radical Res 1997;26(2):113-24.
CBAC COPYRIGHT: CHEM ABS The model quinone compd. menadione has been used to study the effects of oxidative stress in mammalian cells, and to investigate the mechanism of action of the quinone nucleus which is present in many anticancer drugs. The authors used the alk. single cell gel electrophoresis assay (comet assay) to investigate the effects of low doses of this compd. on isolated human lymphocytes. It was found that concns. of menadione as low as 1muM were sufficient to induce strand breaks in these cells. Pre-incubation with the NAD(P)H quinone oxidoreductase inhibitor dicoumarol, enhanced the prodn. of menadione-induced strand breaks. In contrast, the metal ion chelator 1,10-phenanthroline inhibited formation of strand breaks, although prolonged incubation with 1,10-phenanthroline in combination with menadione resulted in an increase in a population of very severely damaged nuclei. A marked variation in the response of lymphocytes from different donors to menadione, and in different samples from the same donor was also obsd.

Yendle JE, Tinwell H, Elliott BM, Ashby J. **The genetic toxicity of time: importance of DNA-unwinding time to the outcome of single-cell gel electrophoresis assays.** Mutat Res 1997;375(2):125-36.
Single-cell gel electrophoresis assays (comet assays) are described in which DNA damage is assessed in mouse skin keratinocytes treated with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and beta-propiolactone (BPL) either in vitro or in vivo. The positive results observed under both conditions of test encourage the further development of the mouse skin comet assay as a screen for direct-acting in vivo genotoxins. From the outset of the present experiments we were struck by the compacted nature of the DNA in mouse skin keratinocytes. Under similar conditions of assay, rodent hepatocytes presented a uniform 'unwound' distribution of DNA over the whole nuclear region. In order to study this effect we varied what seemed to be the most obviously related assay parameter: the DNA-unwinding time. A series of experiments was conducted in which control and MNNG-treated cells were exposed to a range of alkaline DNA-unwinding times (0.3-18 h) followed by measurement of the three comet tail parameters (length, DNA content, and their product, tail moment). Each of these parameters increased with increasing time of unwinding such that the tails observed for MNNG-treated cells with 0.3 h of DNA unwinding were similar in length to the tails of control cells exposed to an 8 h DNA-unwinding time. It is concluded that DNA-unwinding time is a critical parameter of the comet assay and that it may require optimisation for each tissue/cell type studied. Further, the data alert to the prospect that agents that uniquely affect chromosomal protein superstructure may increase comet tail length/DNA content in the absence of chemically induced DNA damage. Thus, there may be two discrete classes of chemical interaction with chromosomal DNA that yield identical comet assay results, but which have different implications for the genetic toxicity of the test agent. Similar effects were observed for rat hepatocytes or mouse lymphoma cells exposed to an 18 h DNA-unwinding time, but no comet tails were produced by exposure of cells to the lysis conditions (pH 10.0) for 18 h.

Yuan W, Kiselyov AS, Harvey RG, Carothers AM. **Mutagenic specificity of syn-benzo(g)chrysene 11,12-dihydrodiol 13,14-epoxide in the dihydrofolate reductase gene of Chinese hamster ovary cells**. Carcinogenesis 1995;16(11):2869-73.
Mutations of the dihydrofolate-reductase (dhfr) gene induced by syn-benzo(g)chrysene-11,12-dihydrodiol-13,14-epoxide (BgCDE) were studied for preference of deoxyadenosine adduct targets. The

parental Chinese-hamster-ovary cell line from which all mutants were generated was hemizygous for the dhfr locus. Cell cultures were treated with 0.75 micromolar BgCDE. Selection for mutants by the tritiated deoxyuridine suicide method followed a 6 day expression period. BgCDE treatment killed 60% of the cell population and induced dhfr negative mutants at a frequency of 9x10(6). Overall, 27 mutations were detected in 26 mutants. At least 89% of the mutations affected purines on the nontranscribed DNA strand. The predominant change was an adenine to thymine transversion. Of the mutations, 59% arose at deoxyadenosine. Targeted bases on exon 4 accounted for 59.3% of the induced changes. The chrysene compound was a much less potent mutagen at the dhfr locus than a previous comparison compound, anti-dihydroxy-epoxy-tetrahydrobenzo(c)phenanthrene. Although both induced point mutations at deoxyadenosine, the target sites and cytotoxicity differed. The authors conclude that preferred mutation target sites within the dhfr gene appear highly agent specific.

Zheng H, Olive PL. **Influence of oxygen on radiation-induced DNA damage in testicular cells of C3H mice.** Int J Radiat Biol 1997;71(3):275-82.
BIOSIS COPYRIGHT: BIOL ABS. Radiation-induced DNA single-strand break (ssb) induction and rejoining were measured in murine testicular cells using the alkaline comet assay. Individual cells in different stages of differentiation were identified on the basis of DNA content. As expected, induction of DNA ssb in testis cells irradiated on ice was independent of ploidy, and the extent of damage was similar to that produced in cells from other normal tissues. However, in vivo irradiation of air-breathing mice produced more ssb in haploid than tetraploid germ cells, although their rates of rejoining were similar and comparable to repair rates of cells from other normal tissues. In addition, irradiation of testis in situ produced only half as much damage as irradiation in vitro, and this could be explained only in part by the rapid ssb rejoining occurring during irradiation and cell isolation. A lower cellular oxygenation was postulated to account for the apparent resistance of testis cells to induction of breaks and the difference in induction in relation to DNA content. This was confirmed when carbogen inhalation and treatment with nicotinamide not only increased the overall degree of ssb induction in all these cells, but also reduced differences.

HEPATIC AND RENAL TOXICITY

Dierickx PJ, Noble E. **Purification and characterisation of the soluble glutathione S-transferase isoenzymes in rat kidney derived NRK cells.** Biochem Mol Biol Int 1997;41(5):1013-23.
Glutathione S-transferase (GST) enzymes are toxicologically important from many points of view. Rat kidney derived established NRK cells were mass cultured for the isolation of GST isoenzymes. These were enriched by affinity chromatography and separated by chromatofocusing and HPLC. Exactly the same major GST subunits were found in NRK cells as in the rat kidney. Strong evidence was also found for the presence of an aberrant form of GST 7-7, as was described in rat kidney. A very good correlation between the NRK GST and rat kidney, and especially cis-platinum treated kidney, was found. It is concluded that NRK cells can be considered as a valuable alternative tool for in vitro research of rat kidney phenomena, especially when toxicological interactions are investigated.

Furitsu H, Ogawara K, Fujita T, Yamashita F, Takakura Y, Sezaki H, Hashida M. **Pharmacokinetics analysis of$_{65}$scavenger receptor-mediated uptake of anionized proteins in the isolated perfused rat**

liver. Int J Pharm 1997;151(1):15-26.

Gonzalez-Martin G, Dominguez AR, Guevara A. **Pharmacokinetics and hepatotoxicity of diclofenac using an isolated perfused rat liver**. Biomed Pharmacother 1997;51(4):170-5.
CBAC COPYRIGHT: CHEM ABS Pharmacokinetics and hepatotoxicity of diclofenac was studied in a recirculating model of isolated perfused rat liver. Ten male Sprague-Dawley rat (weighing 230-330 g) livers were perfused for 2 h with 250 mL Krebs-Henseleit bicarbonate buffer that contained 10.75 mg (group A) and 1.075 mg (group B) of diclofenac (approx. 100 and 10 times the therapeutic dose in man, resp.). Samples were collected from the efflux at regular time intervals for the detn. of diclofenac concns. by a high performance liq. chromatog. (HPLC) method. Pharmacokinetic analyses were carried out using a computer program. To establish viability of the liver and toxicity of the drug, enzyme activity measurements of lactate dehydrogenase (LDH), aspartate aminotransferase (SGOT) and pyruvate aminotransferase (SGPT) were performed by a spectrophotometric method. Oxygen consumption was also recorded during the entire perfusion period. Both groups presented bicompartmental kinetics. Concn. profiles showed that group B had a better metabolizing capacity, reflected in a 85.54 min half-life, a 0.52 mL min-1 g-1 liver clearance and a 0.517 extn. ratio, compared to group A, which presented a 123.95 min half-life, a 0.1164 mL min-1 g-1 liver clearance and a 0.116 extn. ratio. LDH activity showed a significant increase in group A at 90 min in comparison with the control group, while in group B this increase was significantly higher at 10 min. The aminotransferase levels did not show a significant increase. According to these results, diclofenac would not have a direct hepatotoxic effect, even at doses 100 times higher than therapeutic ones.

Guillouzo A, Morel F, Langouet S, Maheo K, Rissel M. **Use of hepatocyte cultures for the study of hepatotoxic compounds.** J Hepatol 1997;26(Suppl 2):73-80.
Human and animal hepatocytes in primary culture are widely used in pharmacotoxicological research. They represent a unique in vitro model since they retain both phase I and phase II enzyme activities as well as their inducibility by xenobiotics. Hepatocyte cultures are used for drug screening, identification of the lesions induced by toxic compounds and determination of mechanisms by which xenobiotics exert liver injury.

Hildebrand H, Hartmann E, Popp A, Bomhard E. **Quantitation of alpha(2)-microglobulin after administration of structurally divergent chemical compounds.** Arch Toxicol 1997;71(6):351-9.
alpha(2)-Microglobulin-induced nephropathy is a phenomenon which is exclusively found in adult male rats. Various chemicals are able to bind to alpha(2)-microglobulin thus inhibiting its proteolytic degradation in lysosomes of the P2 segment of the rat nephron. The accumulation of this protein in 'protein droplets' or 'hyaline droplets' leads to necrosis, followed by regeneration which possibly later results in the formation of tumours. Here we report the development of a monoclonal antibody which is specific for alpha(2)-microglobulin. It was utilized to measure alpha(2)-microglobulin concentrations in plasma and tissues, and to stain alpha(2)-microglobulin in fixed tissue slides. In two studies we administered to adult male Wistar rats two groups of compounds: (1) one group of structurally diverse compounds, which give an overview of chemical entities capable of inducing the accumulation of alpha (2)-microglobulin; and (2) another group of structurally closely related compounds (i.e. substituted benzene derivatives) for the purpose of elucidating possible structure-activity relationships. The degree

of alpha(2)-microglobulin-induced nephropathy was determined by immunohistochemical staining of kidney sections. In addition liver and kidney tissue and plasma concentrations of alpha(2)-microglobulin were not found to be elevated whereas kidney tissue concentrations were higher than the controls. The increase over control values ranged from 154% (1,4-dichloromethyl-benzene) to 321% [alpha-methyl-4-(1-methylethyl)-cyclohexanemethanol]. Comparing structurally related benzene derivatives, the hyaline droplet accumulating (HDA) potential was found to depend both on the type of substituent and its position at the aromatic ring. In general HDA activity increased in the order benzene approximately equal to phenol approximately equal to alkylated phenols < halogenated phenols < halogenated benzenes. Further QSAR studies are needed to provide a theoretical base for these observations.

Ilinskaya ON, Vamvakas S. **Nephrotoxic effects of bacterial ribonucleases in the isolated perfused rat kidney.** Toxicology 1997;120(1):55-63.
Alterations of the renal function in the isolated perfused rat kidney system after application of two bacterial RNases, Bacillus intermedius RNase (binase) and ribonuclease produced by Bacillus amyloliquefaciens (barnase), were investigated with two different treatment regimens in comparison with catalytically inactive derivates of the enzymes, photooxidated at the active site His101 binase and inactive mutant His102Gln barnase. For the in vitro approach the test enzymes were dissolved in the perfusion media and applied to the kidney after removal from the animal. Alternatively, the test ribonucleases were administered to rats in vivo and the renal effects were assessed in the isolated perfused rat kidney 1 and 6 h after treatment. In the in vitro regimen both active enzymes induced time- and concentration-dependent nephrotoxicity reflected in enhancement of urinary protein excretion, decline of glucose reabsorption, increase of gamma-glutamyltranspeptidase and alkaline phosphatase activities in urine. In vivo administration of active binase induced functional impairment of the isolated perfused organ in a similar way. None of the inactive RNases in both regimens and at all concentrations tested altered any renal parameter. The results suggest that RNA degradation may be involved in the nephrotoxic effects of bacillar RNases.

Indulski JA, Lutz W. **[Biomarkers of hepatotoxic effects: their usefulness in occupational medicine]**. Med Pr 1997;48(2):177-87. (Pol)
Medical screening and the resultant monitoring of health effects induced by hepatotoxins present in workplaces become of still greater importance in the assessment of occupational health and safety. Health effects of occupational and nonoccupational hepatotoxic factors may be acute, subacute or chronic. Laboratory tests (biomarkers) used in screening for detection of asymptomatic damages of the liver should satisfy the following three criteria: 1. they should provide positive or negative predictive information, namely the information about possible development of clinically evident hepatopathy; 2. they should be very sensitive and specific in order to ensure a correct identification of the developing disease; and 3. they should provide information which of clinical biomarkers should be applied subsequently in order to confirm and facilitate the diagnosis of hepatopathy related to exposure to occupational hepatotoxins or to eliminate such a relationship. It should be also remembered that for assessing disorders in hepatic functions those biomarkers should be selected which are most effective in identifying both persons with hepatopathy induced by environmental hepatotoxins and those who are free from the liver damages. It should be stressed that to date none of the existing biomarkers is sensitive and specific enough to assess alone all the functional systems of the liver.

Kawabata T, Ma Y, Yamador I, Okada S. **Iron-induced apoptosis in mouse renal proximal tubules after an injection of a renal carcinogen, iron-nitrilotriacetate.** Carcinogenesis 1997;18(7):1389-94.
Redox-active iron was demonstrated in mouse kidney by Timm's sulphide-silver staining after an injection of a renal carcinogen, iron-nitrilotriacetate (Fe-NTA). The iron was on the apical site of tubular epithelia of the renal proximal convoluted portion and in the tubules of the straight portion 30 min after the Fe-NTA injection. As the epithelial cells of the proximal tubules died, the iron disappeared in the dead cells and was stored in the cytoplasm of the more distal tubular epithelia. Biochemically, redox-active iron in the kidney rapidly increased to four times higher than the control 30 min after the Fe-NTA injection, then decreased to a plateau which was still higher than the control. Iron tightly stored in iron-storage proteins increased gradually by 3 h after the injection and then decreased at 5 h. The iron-induced free radical injuries, such as lipid peroxidation and protein oxidation, were demonstrated in the renal proximal tubules by histochemistry. The nuclei of the proximal tubular epithelia shrank and fragmented with the free radical injuries, and were positive for terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labelling. DNA ladder was demonstrated in the mice renal cortexes by agarose gel electrophoresis. It was elucidated that redox-active iron caused free radical injuries in the proximal tubules of mice kidneys after the injection of a renal carcinogenic iron (Fe-NTA) and induced the apoptosis of the proximal tubular epithelial cells.

Kwon Y, Morris ME. **Membrane transport in hepatic clearance of drugs I: extended hepatic clearance models incorporating concentration-dependent transport and elimination processes**. Pharm Res 1997;14(6):774-9.
CBAC COPYRIGHT: CHEM ABS The objective of the present study was to develop hepatic clearance models which incorporate a unidirectional carrier-mediated uptake and bidirectional diffusional transport processes for drug transport in the sinusoidal membrane of hepatocytes as well as nonlinear intrinsic elimination. Two models were derived which view the liver as two sep. compartments, i.e., sinusoid and hepatocyte. Model I assumes the instantaneous complete mixing of drugs within each compartment (similar to that of the well-stirred model), while model II assumes that the drug concns. in both compartments decrease progressively in the direction of the hepatic blood flow path (similar to that of the parallel-tube model). Computer simulations were performed using a range of steady-state infusion rates for a substrate, while varying the Vmax (capacity) and Km (Michaelis-Menten const.) for the carrier-mediated uptake process, the diffusional clearance, the Vmax and Km for the intrinsic elimination process, blood flow and protein binding. Simulations in which Vmax and Km for the sinusoidal membrane transporter and the diffusional clearance were varied, demonstrated that these membrane transport processes could affect the clearance of drugs to a significant extent in both models. The ests. for clearance of substrates with the same pharmacokinetic parameters are always lower in model I than in model II, although the quant. differences in parameter ests. between models varied, depending on the steady state infusion rates. These more general hepatic clearance models will be most useful for describing the hepatic clearance of hydrophilic compds., such as org. anions or cations, which exhibit facilitated uptake and limited membrane diffusion in hepatocytes.

Lewis DF, Lake BG. **Quantitative structure-activity relationship (QSAR) analysis for a series of rodent peroxisome proliferators: interaction with the mouse liver peroxisome proliferator-**

activated receptor alpha (mPPARalpha). Toxicol In Vitro 1997;11(1-2)99-105.
CBAC COPYRIGHT: CHEM ABS The results of quant. structure-activity relationship (QSAR) anal. on a structurally diverse group of peroxisome proliferators are reported. The relative potencies of 11 peroxisome proliferators(with respect to clofibric acid) for induction of palmitoyl-CoA oxidn. in rat hepatocyte cultures appear to be detd. by a combination of lipophilicity (logP descriptor) and calcd. binding affinity (logK) to a model of the mouse liver peroxisome proliferator-activated receptor alpha (mPPARalpha) ligand-binding domain. It is possible that desolvation of the putative binding site and ligand ionization may also play a role in activation of the mPPARalpha.

Rogiers V, Vandenberghe Y, Vanhaecke T, Geerts A, Callaerts A, Carleer J, Roba J, Vercruysse A. **Observation of hepatotoxic effects of 2-n-pentylaminoacetamide (Milacemide) in rat liver by a combined in vivo/in vitro approach.** Arch Toxicol 1997;71(5):271-82.

Roma MG, Orsler DJ, Coleman R. **Canalicular retention as an in vitro assay of tight junctional permeability in isolated hepatocyte couplets: effects of protein kinase modulation and cholestatic agents.** Fundam Appl Toxicol 1997;37(1):71-81.
A simple, fast method to evaluate acute changes of tight junctional permeability in isolated hepatocyte couplets is proposed. The method consists of the recording of the number of canalicular vacuoles able to retain the previously accumulated fluorescent bile acid analogue cholyl-lysyl-fluorescein (CLF), as visualized by inverted fluorescent microscopy, following acute exposure to the compounds under study. The method was validated by (i) making a systematic documentation of the effect on CLF retention of a variety of hormonal modulators (vasopressin and phorbol esters), as well as several cholestatic (taurolithocholic acid, cyclosporin A, and estradiol 17 beta-glucuronide) and hepatotoxic agents (menadione, A23187, and t-butyl hydroperoxide), all known to affect biliary permeability in intact liver, and (ii) carrying out a comparative analysis of the results obtained with those recorded using rapid canalicular access of horseradish peroxidase (HRP) as an alternative procedure. The compounds tested all decreased canalicular vacuolar retention of CLF in a dose-dependent manner. Vasopressin- and phorbol ester-induced decline in CLF retention were prevented by pretreatment with the protein kinase C inhibitors H-7 and staurosporine, thus confirming a role for this enzyme in canalicular permeability regulation. A significant direct correlation ($r = 0.934$, $p < 0.001$) was obtained when the decrease in canalicular retention of CLF was compared with the increment in the canalicular access of HRP. Image analysis revealed that cellular fluorescence was not increased following exposure to these compounds, suggesting a paracellular rather than transcellular route for CLF egress. These results all support canalicular vacuolar retention of CLF as a suitable method to readily evaluate acute changes in tight junctional permeability in isolated hepatocyte couplets induced by physiological modulators or hepatotoxic agents.

IMMUNOTOXICITY

Ashby J, Basketter DA, Paton D, Kimber I. **Structure activity relationships in skin sensitization using the murine local lymph node assay**. Toxicology 1995;103(3):177-94.
Major classes of contact allergens were identified using a murine local lymph node assay. Mice were exposed to various concentrations of 106 test chemicals on the dorsum of each ear for 3 days, then

injected intravenously with tritiated thymidine. Activity was measured as a function of isotope incorporation in draining auricular lymph nodes. The sensitizing activity observed for 106 chemicals was segregated into active and inactive agents on the basis of producing a three fold or greater increase in proliferative activity. The allergens were then segregated into six groups: electrophiles, potential electrophiles following metabolism, potential Michael reactive agents, benzoylating agents, ionic chemicals, and other. In the lymph node assay, 73 chemicals were active. Broad structure activity relationships (SAR) and quantitative SAR (QSAR) for skin sensitization were determined for the six classes. Electrophiles were segregated into eight chemical subgroups: alkyl halides, sulfonates, sulfates, nitrosamides and nitrosoguanides, aromatic alkyl halides, aromatic alkylating agents, acylating agents, and miscellaneous. Potential electrophiles fell into the groups: epoxide and aromatic nitro/amino compounds. For potential Michael reactive agents, the Elman reaction could determine a valuable QSAR. For ionic chemicals, ionic/acidic functions within a molecule triggered local lymph responses, although acidity alone was ineffective. Benzoylating agents provided an alert to sensitization, but the SAR was based on three analogues. SAR were based on defining major structural alerts involved in contact sensitization, but lack of key data prevented discernment of QSAR. The authors conclude that skin sensitization is elicited by a variety of chemical interactions and a variety of parallel SAR and QSAR will eventually be developed.

Homey B, Schuppe H, Assmann T, Hans-Werner V., Lauerma AI, Ruzicka T, Lehmann P. **A local lymph node assay to analyze immunosuppressive effects of topically applied drugs**. Eur J Pharmacol 1997;325(2-3):199-207.
CBAC COPYRIGHT: CHEM ABS Topical glucocorticosteroids represent the mainstay of antiinflammatory therapy in the treatment of inflammatory skin diseases. Their clin. use, however, is limited by local and systemic side-effects. Thus, in dermatopharmacol., there is a large demand for alternative non-steroidal antiinflammators. Other than transplantation models, most of the frequently used in vivo test systems for assessment of drug-induced immunosuppression measure changes in inflammatory skin responses by means of skin erythema and edema after challenge of sensitized animals. The aim of this study was to develop an alternative mouse model to detect and analyze immunosuppressive effects of topically applied drugs. On the basis of a modified local lymph node assay, we analyzed effects of topical hydrocortisone, dexamethasone, mometasone furoate and FK506 (tacrolimus) during the induction phase of contact hypersensitivity. On 4 consecutive days, NMRI mice were treated on the dorsal surfaces of both ears with increasing concns. of test compd. During the last 3 days, the mice received in addn. the contact sensitizer, oxazolone (1%). On day 5, draining auricular lymph nodes were removed in order to assess lymph node cell counts and perform flow cytometric anal. of lymph node cell subpopulations (CD4+/CD25+, Ia+/CD69+, Ia+/B220+). All test compds. proved to exert significant immunosuppressive effects after topical application, but showed differences in their immunomodulatory potential. In conclusion, the local lymph node assay serves as an appropriate model to characterize immunosuppressive effects of topically applied drugs by measuring immunol. relevant end-points.

Hurks HM, Out-Luiting C, Vermeer BJ, Claas FH, Mommaas AM. **In situ action spectra suggest that DNA damage is involved in ultraviolet radiation-induced immunosuppression in humans.** Photochem Photobiol 1997;66(1):76-81.

The mixed epidermal cell lymphocyte reaction (MECLR) is a commonly used method to study the immunomodulatory effects of UV radiation. The in vitro action spectrum for the MECLR showed that the UV-induced suppression of the MECLR responses is associated with UV-induced DNA damage. To investigate whether in vivo DNA damage also leads to the abrogation of the MECLR, in situ action spectra were made for the MECLR and the induction of thymine dimers (T < > T). Human skin, obtained from plastic surgery, was exposed to monochromatic light of 254, 297, 302 and 312 nm. After irradiation, epidermal cells were isolated and used as stimulator cells in the MECLR or processed for flow cytometric detection of T < > T. On the basis of dose-response curves for each wavelength, the action spectra for suppression of the MECLR and the induction of T < > T were calculated. These spectra showed close similarities, suggesting that, also in situ, UV-induced DNA damage is involved in the UV-induced suppression of the MECLR. Both action spectra showed a small decline from 254 nm to 302 nm, followed by a steep decline to 312 nm. These data show that, in situ, UVC can efficiently induce DNA damage and modulate cutaneous immune responses.

Kalish RS, Wood JA, Kydonieus A, Wille JJ. **Prevention of contact hypersensitivity to topically applied drugs by ethacrynic acid: potential application to transdermal drug delivery**. J Controlled Release 1997;48(1):79-87.
CBAC COPYRIGHT: CHEM ABS Transdermal drug delivery systems have many advantages. However, the extension of this technol. to addnl. drugs is limited by the development of contact sensitization to topically applied drugs. Ion channel modulators have been reported to inhibit elicitation of allergic contact sensitivity. The authors developed a mouse ear swelling test (MEST) for sensitization to topically applied nadolol, albuterol, clonidine, and chlorpheniramine. Pre-treatment of the backs of mice with ethacrynic acid (0.5%), prevents subsequent sensitization from topical application of the above drugs. Ethacrynic acid must be applied prior to the sensitizing drug, and the effects of ethacrynic acid are specific for the site of application. Skin permeation studies detd. that redn. in sensitization was not the result of redn. in drug penetration. Ethacrynic acid-induced inhibition does not induce a generalized immunosuppression as the mice can be sensitized to drugs or haptens applied to other sites. It is proposed that counter-sensitizers can be incorporated into transdermal drug delivery systems to prevent sensitization, and expand the application of this technol. to potentially sensitizing drugs.

Mansfield E, Chiron MF, Amlot P, Pastan I, Fitzgerald DJ. **Recombinant RFB4 single-chain immunotoxin that is cytotoxic towards CD22-positive cells.** Biochem Soc Trans 1997; 25(2):709-14.

Nishijima T, Tokura Y, Imokawa G, Seo N, Furukawa F, Takigawa M. **Altered permeability and disordered cutaneous immunoregulatory function in mice with acute barrier disruption.** J Invest Dermatol 1997;109(2):175-82.
In vivo and in vitro T-cell-activating ability of murine epidermal cells (EC) was investigated in acutely barrier-disrupted skin by extraction of epidermal lipids with acetone or removal of corneocytes by tape stripping. Contact sensitivity (CS) to 2,4-dinitrofluorobenzene (DNFB) and picryl chloride (PCl) and contact photosensitivity (CPS) to tetrachlorosalicylanilide (TCSA) were significantly augmented when challenged or sensitized at sites treated with acetone 24 h before, compared with the intact skin. CS to DNFB was also enhanced by tape stripping, but not by water rubbing, suggesting that physical stress or a toxic effect of acetone was not responsible for the augmentation. Semi-quantification of TCSA-EC

photoadducts showed markedly increased permeability of hapten in the epidermis 24 h after acetone treatment. Bioactive IL-1alpha was more pronounced in barrier-disrupted than in intact skin. Lymph node T cells from PCl-sensitized mice proliferated significantly more in a hapten-specific and co-stimulatory molecule-dependent manner in response to trinitrophenylated (TNP) EC from acetone-treated skin than to those from untreated skin. Immunofluorescence staining of epidermal sheets and flow cytometric analysis of dispersed EC showed that subpopulations of Langerhans cells (LC) in acetone-rubbed or tape-stripped skin expressed major histocompatibility complex class II CD54 and CD86 molecules at levels higher than the rest of LC and LC from water-treated or untreated epidermis. Therefore, not only increased permeability of hapten through the epidermis but also altered immune functions of EC potentiate T-cell activation in acute barrier disruption. Such augmentation of immune reactivity may be critical to elimination of environmental noxious agents that penetrate easily into the barrier-disrupted epidermis.

Qu M, Muller HK, Woods GM. **Chemical carcinogens and antigens contribute to cutaneous tumor promotion by depleting epidermal Langerhans cells.** Carcinogenesis 1997;18(6):1277-9.
Epidermal Langerhans cells (LC) are an integral component of the skin immune system as they initiate immune responses to a variety of antigens, including tumor antigens. When skin is exposed to carcinogenic doses of ultraviolet-B irradiation, chemical carcinogens or tumor promoters there is a significant reduction of LC density. This causes the skin to be immunocompromised and provides an opportunity for aberrant cells to escape immune detection and develop into tumors. Consequently LC depletion is a key event associated with the pathogenesis of skin cancer. We propose that LC depletion contributes to tumor promotion and therefore any agents that reduce LC number, e.g. the contact sensitizing antigen 2,4,6-trinitrochlorobenzene (TNCB), may also contribute to tumor promotion. This proposal was evaluated in cutaneous carcinogenesis by treating mouse skin with a tumor initiating dose of the carcinogen 7,12-dimethylbenz[a]anthracene (DMBA) followed by a tumor promoter. The initiating dose of DMBA did not cause LC depletion or tumor development. However, if the DMBA-treated skin was then exposed to a concentration of TNCB that caused LC depletion, skin tumors developed. This is analogous to the classical initiator/promoter system with an LC-depleting dose of TNCB contributing to tumor promotion. Further, this promotion effect was independent of the commencement time of the promoter application, as 2% TNCB applied either 1 or 12 weeks after DMBA initiation induced tumor development. Analysis of the association of LC depletion with immunosuppression and tumor promotion, showed that these events were linked, irrespective of the agent that caused the depletion. It is therefore concluded that LC depletion and local immunosuppression are important aspects of tumor promotion in cutaneous carcinogenesis and non-carcinogenic agents may have tumor promoter activities.

Roscoe DM, Pai LH, Pastan I. **Identification of epitopes on a mutant form of Pseudomonas exotoxin using serum from humans treated with Pseudomonas exotoxin containing immunotoxins.** Eur J Immunol 1997;27(6):1459-68.
PE38 is a 38-kDa derivative of the 66-kDa Pseudomonas exotoxin (PE) in which the cell binding domain of PE (domain Ia, amino acids 1-252) and a portion of domain Ib (amino acids 365-380) are deleted. The immunotoxins LMB-1 and LMB-7 contain PE38 and kill cancer cells by exploiting the cytotoxic action of PE38. The major human B cell epitopes of PE38 were mapped by measuring the

reactivity of 45 serum samples from patients treated with the PE38-containing immunotoxins LMB-1 or LMB-7 to two panels of overlapping synthetic peptides representing the sequence of PE38. One panel of peptides is ten amino acids long and overlap by seven amino acids, and the second panel of peptides is twenty amino acids long and overlap by ten. Five major epitopes were identified: amino acids 274-283, 470-492, 531-540, 555-564, and the C-terminal amino acids 596-609. Two minor epitopes were identified as well: amino acids 501-510 and 582-589. These epitopes are predominantly located on the surface of the protein. The amino acids believed to be critical for binding are highly solvent-accessible residues. The results of the human antibody response to peptides are compared to the pattern of reactivity previously identified with serum samples obtained from monkeys.

Vos JG, Van Loveren H. **Markers for immunotoxic effects in rodents and man**. Toxicol Lett 1995;82-83:385-94.
Models used to assess the immunotoxicity of chemical compounds in experimental animals and humans were described and discussed. Recently revised guidelines for immunotoxicity testing in the Netherlands were described. Once a chemical has been identified as being immunotoxic, assessment of immunologic function and no adverse effect levels was recommended. A tiered approach for the assessment of immunotoxicity used by the United States National Toxicology Program has included evaluation of hematology, lymphoid organ weight, splenic cellularity and histology, the thymus, and lymph nodes as well as an in-vitro splenic immunoglobulin-M (IgM) plaque forming assay, in-vitro analysis of lymphocyte proliferation following stimulation, and in-vitro assessment of natural killer cell activity. The highest accuracy for identification of potential immunotoxicity was seen with the use of the splenic IgM plaque forming cell response and cell surface marker analysis. Animal models of autoimmune disease were described; the application of such models to the assessment of the exacerbation of autoimmunity by chemical exposures has not been studied. The popliteal lymph node assay has had promising results for use in the evaluation of chemicals that may induce autoimmune like responses. Immunotoxicity assessment in humans has generally been conducted by the use of cross sectional studies examining certain biomarkers following exposure to a suspected immune system toxin. The most common biomarkers of exposure have included chemical levels in urine, blood, or target organ or tissue and the identification of specific antibodies or positive skin tests. Functional changes in immune cells have been used as a biomarker of chemical effects and genetic analyses have been used as markers of susceptibility. Methods for extrapolation of experimental data to humans were reviewed as were several known immunotoxins including polychlorinated-hydrocarbons and hexachlorobenzene (118741).

Wu M. **Enhancement of immunotoxin activity using chemical and biological reagents.** Br J Cancer 1997;75(9):1347-55.
One of the major discoveries of effective therapeutics is the use of targeted treatment, such as antibody-directed toxins, i.e. immunotoxins; however, this medicine delivery strategy is still at a developmental stage. A number of problems need to be resolved; one is their inefficacy when applied in vivo. Research has stimulated interest in this area through the use of chemical reagents and other moieties to increase the activity of immunotoxins. In this article, reagents that can potentiate the cytotoxicity of immunotoxins are reviewed and the mechanisms that increase activity of immunotoxins are discussed. Lysosomotropic amines, especially ammonium chloride and chloroquine, may raise the pH value of the lysosome in which the conjugates enter. Carboxylic ionophores, e.g. monensin, can influence Golgi

vacuolation, which may facilitate the routing of conjugates, augmenting activity. Calcium channel antagonists may increase immunotoxin killing through morphological or other mechanisms that are not yet well understood. Viral particles and surface structure can enhance the cytotoxicity of conjugates, probably through the mechanism of disrupting endosomes. In addition, cytokines, beta-adrenergic blockers, immunosuppressive agents (cyclosporin A) and some antibiotics (daunorubicin) can be used to increase the effect of immunotoxins.

NEUROTOXICITY

Barnham KJ, Dyke TR, Kem WR, Norton RS. **Structure of neurotoxin B-IV from the marine worm Cerebratulus lacteus: a helical hairpin cross-linked by disulphide bonding.** J Mol Biol 1997;268 (5):886-902.
B-IV is a 55-residue, crustacean-selective, neurotoxin secreted by Cerebratulus lacteus, a large marine worm found along the northeastern coast of North America. The 3D structure of this molecule in aqueous solution has been determined by 1H NMR.

Brandenburg CA, May V, Braas KM. **Identification of endogenous sympathetic neuron pituitary adenylate cyclase-activating polypeptide (PACAP): depolarization regulates production and secretion through induction of multiple propeptide transcripts**. J Neurosci 1997;17(11):4045-55.

Britt JC, Brenner HR. **Rapid drug application resolves two types of nicotinic receptors on rat sympathetic ganglion cells**. Pfluegers Arch 1997;434(1):38-48.

Goto K, Mochizuki H, Hattori T, Nakamura N, Mizuno Y. **Neurotoxic effects of papaverine, tetrahydropapaverine and dimethoxyphenylethylamine on dopaminergic neurons in ventral mesencephalic-striatal co-culture.** Brain Res 1997;754(1-2):260-8.
We report neurotoxic effects of papaverine, tetrahydropapaverine, dimethoxyphenylethylamine (DMPEA), and 1-methyl-4-phenylpyridinium ion (MPP+) on dopaminergic neurons in ventral mesencephalic-striatal co-culture. These compounds have been reported as mitochondrial toxins which may be implicated in the etiology and pathogenesis of Parkinson's disease. Tyrosine hydroxylase (TH)-positive neurons were decreased in dose-dependent manner by these compounds. Papaverine and MPP+ were most toxic to TH-positive neurons among the compounds tested. The order of the toxicity on TH-positive neurons was papaverine, MPP+, tetrahydropapaverine and then DMPEA. This order of toxicity was approximately the same as that reported on the inhibitory effect of these compounds on NADH-linked mitochondrial respiration and complex I activity. These findings indicate that the presence of dimethoxy residues in the catechol ring augments toxicity to dopaminergic neurons in culture.

Hammarlund-Udenaes M, Paalzow LK, De Lange EC. **Drug equilibration across the blood brain barrier--pharmacokinetic considerations based on the microdialysis method**. Pharm Res 1997;14:128-34.

Jirsova K, Mandys V. **Carboplatin-induced micronuclei formation in non-neuronal cells of rat**

foetal dorsal root ganglia cultured in vitro and comparison with another anticancer drug--cisplatin. Sb Lek 1996;97(3):331-42.
Carboplatin-induced chromosomal damage was evaluated in two types of non-neuronal cells, fibroblasts and Schwann cells, migrating from rat foetal dorsal root ganglia (DRG) in explant cultures by quantification of micronuclei (MN). Evaluation of granular condensation of nuclear chromatin, another toxicological phenomenon, closely related to activation of apoptosis, was performed to compare genotoxic and cytotoxic action of the drug. Both changes were dependent upon the concentration of the drug as well as on the exposure time. Dose-response curves for micronuclei in fibroblasts revealed normal distribution with the maximum at 100, 25 and 5 mumol/l after 24, 48 and 72 hours treatment of carboplatin, respectively. The maximum number of micronuclei in Schwann cells was obtained at 25, 25 and 12.5 mumol/l at the same exposure time. Micronucleation of fibroblasts represented 293, 382 and 376% of control values and in Schwann cells 366, 819 and 1667%, respectively. These results were compared with the data obtained in our previous experiments where identical cell types were influenced by cisplatin. The maximal micronucleation in fibroblasts treated with cisplatin reached 208-385% of control values at the same intervals. The maximal number of micronuclei in Schwann cells was induced by cisplatin at the dosis about 20 times lower compared to carboplatin (ranged from 2 to 0.75 mumol/l) and represented 914, 1032, 1693% of control values. Our results suggest that Schwann cells are more sensitive to platinum drugs than fibroblasts, especially to cis-DDP. Moreover, carboplatin revealed delayed toxic effect in comparison with cisplatin. This finding could contribute to the explanation of different neurotoxic potential of both cytostatics.

Kim CS, Binienda Z, Sandberg JA. **Construction of a physiologically based pharmacokinetic model for 2,4-dichlorophenoxyacetic acid dosimetry in the developing rabbit brain**. Toxicol Appl Pharmacol 1996;136(2):250-9.
As part of a larger program intended to extend a physiologically based pharmacokinetic (PBPK) dosimetry model of 2,4-dichlorophenoxyacetic-acid (94757) (2,4-D) to the entire gestation period, a PBPK model was developed for the fetal rabbit brain at near term pregnancy. The model had material compartments including arterial and venous blood, brain, and the rest of the body (with central and deep compartments). The fetus model had compartments including brain tissue, brain plasma, cerebrospinal fluid, body, amniotic fluid, and arterial and venous blood. 2,4-D was carried by the maternal blood to the placenta, where the 2,4-D diffused into the fetal circulation. The model was used to predict maternal and fetal concentrations of 2,4-D following administration of 2,4-D at dose levels of 1, 10, or 40mg/kg to pregnant rabbits intravenously. The brain uptake was membrane limited by the blood/brain barrier with saturable clearance from the cerebrospinal fluid into the venous blood by the choroid plexus in both fetus and mother. The authors conclude that the model adequately simulated the 2 hour time course of 2,4-D concentrations in both mother and fetus, based on experimental data. This generic PBPK model should be a useful tool for evaluating the safety or organic acid neurotoxicants in the developing brain.

Maruyama W, Naoi M, Kasamatsu T, Hashizume Y, Takahashi T, Kohda K, Dostert P. **An endogenous dopaminergic neurotoxin, N-methyl-(R)-salsolinol, induces DNA damage in human dopaminergic neuroblastoma SH-SY5Y cells.** J Neurochem 1997;69(1):322-9.

Sanz-Rodriguez C, Boix J, Comella JX. **Cytosine arabinoside is neurotoxic to chick embryo spinal**

cord motoneurons in culture. Neurosci Lett 1997;223(3):141-4.
Cytosine arabinoside (1-beta-D-arabinofuranosylcytosine, AraC) is a commonly used antimitotic agent that kills proliferating cells by inhibiting DNA synthesis. We report that AraC is toxic to cultured chick embryo spinal cord motoneurons (MTNs) in a concentration-dependent fashion with an EC50 of about 2 microM. Interestingly, this type of MTN death is specific, resembles that occurring upon muscle extract (MEX) trophic deprivation regarding its morphological and temporal characteristics, and has apoptotic features, as judged by observation of nuclear morphology. The death of AraC-treated MTNs can be blocked by 2'-deoxycytidine (dC), a pyrimidine metabolite AraC is structurally related to. Overall, these findings suggest that dC may participate in a pathway, different from inhibition of DNA synthesis, that is necessary for cultured MTNs to respond to the trophic activities present in MEX.

Schwarz R, Callahan M, Davis R, Jaen J, Tecle H. **Development of M1-subtype-selective muscarinic agonists for Alzheimer's disease: translation of in vitro selectivity into in vivo efficacy**. Drug Dev Res 1997;40(2):133-43.

Seeger G, Hartig W, Rossner S, Schliebs R, Bruckner G, Bigl V, Brauer K. **Electron microscopic evidence for microglial phagocytic activity and cholinergic cell death after administration of the immunotoxin 192IgG-saporin in rat.** J Neurosci Res 1997;48(5):465-76.
192IgG-saporin represents a novel cholinergic immunotoxin which selectively and specifically destroys cholinergic cells in rat basal forebrain. Activated microglial cells are known to play an important role in phagocytosis in regions of neuronal loss. To study the immunotoxin-induced phagocytic events in the basal forebrain activated microglial cells were visualized by lectin cytochemistry using Griffonia simplicifolia agglutinin and analyzed by electron microscopy. Three and 7 days following an intracerebro-ventricular injection of 4 microg 192IgG-saporin, increased numbers of activated microglial cells were observed at both survival times, but the number was strikingly increased at day 7 postlesion. Three days after immunotoxin application microglial cells displayed features similar to those of resting microglia. Only translucent vacuole-like hollows were found intracellularly beneath the plasma membrane of microglial cells and in the adjoining extracellular space. Most neurons in the vicinity of microglial cells did not show any signs of degeneration. However, 7 days after injection of the immunotoxin microglial cells revealed different stages of phagocytosis. The majority of microglial cells were localized in perineuronal positions attached by processes to large areas of neuronal soma or dendrites, which in general showed signs of severe degeneration. The present study provides electron microscopic evidence for phagocytic microglial reactions in the rat basal forebrain after cholinergic lesion by 192IgG-saporin.

Vardhan KS, Rudra MP, Rao SL. **Inhibition of tyrosine aminotransferase by beta-N-oxalyl-L-alpha, beta-diaminopropionic acid, the Lathyrus sativus neurotoxin.** J Neurochem 1997;68(6):2477-84.
BIOSIS COPYRIGHT: BIOL ABS. Species differences in susceptibility are a unique feature associated with the neurotoxicity of beta-N-oxalyl-L-alpha,beta-diaminopropionic acid (L-ODAP), the Lathyrus sativus neurotoxin, and the excitotoxic mechanism proposed for its mechanism of toxicity does not account for this feature. The present study examines whether neurotoxicity of L-ODAP is the result of an interference in the metabolism of any amino acid and if it could form the basis to explain the species differences in susceptibil Thus, Wistar rats and BALB/c (white) mice, which are normally resistant to L-

ODAP, became susceptible to it following pretreatment with tyrosine (or phenylalanine), exhibiting typical neurotoxic symptoms. C57BL/6J (black) mice were, however, normally susceptible to L-ODAP without any pretreatment with tyrosine. Among the various enzymes associated with tyrosine metabolism examined, the activity of only tyrosine aminotransferase (TAT) was inhibited specifically by L-ODAP. The inhibition was noncompetitive with respect to tyrosine (Ki = 2.0 : 0.1 mM) and uncompetitive with respect to alpha-ketoglutarate (Ki = 8.4 : 1.5 mM). The inhibition of TAT was also reflected in a marked decrease in the rate of oxidation of tyrosine by liver slices, an increase in tyrosine levels of liver, and also a twofold increase in the dopa and dopamine contents of brain in L-ODAP-injected black mice. The dopa and dopamine contents in the brain of only L-ODAP-injected white mice did not show any change, whereas levels of these compounds were much higher in tyrosine-pretreated animals. Also, the radioactivity associated with tyrosine, dopa, and dopamine arising from (14C)tyrosine was twofold higher in both liver and brain of L-ODAP-treated black mice. Thus, a transient increase in tyrosine levels following the inhibition of hepatic TAT by L-ODAP and its increased availability for the enhanced synthesis of dopa and dopamine and other likely metabolites (toxic?) resulting therefrom could be the mechanism of neurotoxicity and may even underlie the species differences in susceptibility to this neurotoxin.

Wang Xingmin, Meng Xiaoqi, Wang Chenghuai. **[Polymerase chain reaction for detection of type A botulinal neurotoxin gene and identification of Clostridium botulinum type A]**. Zhonghua Weishengwuxue He Mianyixue Zazhi 1997;17(3):176-81. (Chi)
BIOSIS COPYRIGHT: BIOL ABS. Botulism is a neuroparalytic disease caused by the neurotoxin produced from Clostridium botulinum. The rapid diagnosis and typing of botulism is significant for the treatment of botulism and decreasing the mortality. In this study,a polymerase chain reaction (PCR) was developed for detection of neurotoxin gene of Clostridium botulinum type A using a set of oligonucleotide primer which designed from the nucleotide sequence of the light chain of type A neurotoxin gene to amplify a fragment of 472bp. Sixty eight strains belonging to 10 clostridial species were detected by the PCR. The results revealed that all of the 20 strains of Clostridium botulinum type A were positive and the others were all negative in PCR. The fragments amplified from several strains of Clostridium botulinum type A were digested with restriction endonuclease to identify the amplified products,and the digestion patterns were in agreement with the sizes estimated from the sequence data. Clear fragment could be obtained from 310pg of DNA could be detected with phenol-chloroform extracts in this PCR. Therefore,it suggests that the PCR system is specific and sensitive for identification of Clostridium botulinum type A and rapid diagnosis of type A botulism.

Widdowson PS, Gyte A, Simpson MG, Wyatt I, Lock EA. **Changes in cerebellar amino acid neurotransmitter concentrations and receptors following administration of the neurotoxin L-2-chloropropionic acid**. Toxicol Appl Pharmacol 1996;136(1): 57-66.
The neurochemical changes resulting from the damage and loss of granule cells following exposure to L-2-chloropropionic-acid (29617661) (LCPA) was examined in rats. Male Alderly-Park-rats were administered LCPA orally at 250mg/kg/day for 3 days, and were terminated from 12 to 120 hours after exposure. Brain tissue was analyzed for amino acid and receptor concentrations. A substantial loss in granule cells and a marked swelling of the cerebellum were observed in rats terminated 5 days after the first dose of LCPA. Glutamate and aspartate concentrations decreased over time in rats treated with

LCPA. At 60 to 84 hours after the first LCPA dose, a 75% decrease in aspartate concentration and a 30% decrease in glutamate concentration were found. The taurine concentration decreased after 78 hours to 66% of the level observed in controls. Concentrations of glutamine, gamma-aminobutyric-acid (GABA), and glycine increased significantly after LCPA treatment. Maximal increases in glutamine and GABA concentrations were about 300% and 400%, respectively, of control values. These disturbances were not evident in forebrain tissue samples. Five days after the initiation of LCPA administration, densities of the N-methyl-D-aspartate (NDMA) and kainate receptors were significantly decreased in the cerebellar cortex, compared to controls. Receptor densities were not reduced in other brain regions. The quantities of GABA and adenosine-A1 receptors were not significantly different between the experimental and control groups. The authors conclude that LCPA exposure causes extensive and selective damage to the neurochemicals of the cerebellum.

Zamani MR, Allen YS, Owen GP, Gray JA. **Nicotine modulates the neurotoxic effect of beta-amyloid protein(25-35)) in hippocampal cultures.** Neuroreport 1997;8(2):513-7.
Two major features of Alzheimer's disease (AD) are beta-amyloid protein (beta AP) deposition and a severe cholinergic deficit. An association between the two is suggested by the negative correlation found between cigarette smoking and AD. We sought to investigate this further by examining the effects of acute and chronic nicotine exposure on beta AP-induced neuronal loss in rat hippocampal cultures. Nicotine was found to attenuate the neurotoxicity of higher concentrations of beta AP(25-35), an effect which was enhanced by longer nicotine pretreatment and significantly inhibited by the nicotine receptor antagonist mecamylamine. Our results suggest that nicotine partially protects against the neurotoxic actions of beta AP(25-35) via a receptor-mediated pathway.

OCULAR TOXICITY

Barratt MD. **QSARS for the eye irritation potential of neutral organic chemicals.** Toxicol In Vitro 1997;11(1-2):1-8.
BIOSIS COPYRIGHT: BIOL ABS. A quantitative structure-activity relationship (QSAR) was derived previously relating European Community (EC) eye irritation classification data of a set of neutral organic chemicals, to log(octanol/water partition coefficient), to the minor principal inertial axes (Ry and Rz) and to dipole moment. Eye irritation scores on a scale of 1-10 for a set of aliphatic alcohols (from the work of Smyth and Carpenter) have been shown to correlate well with the same four physicochemical parameters by means of neural network analysis. The original classification dataset of neutral organic chemicals has been augmented by the addition of a number of the aliphatic alcohols from the Smyth and Carpenter data that could unequivocally be assigned the EC classifications of irritant (those with eye irritation scores of 8 and 9) or non-irritant (scores of 1). Analysis of the extended dataset by both principal components and neural network analysis showed a clear discrimination between irritant and non-irritant chemicals using the same four physicochemical parameters. Predictions of EC eye irritation classifications for aliphatic alcohols with eye scores of 2-7, using the neural network model, showed that alcohols with eye scores of 2 and 3 lie on the classification boundary between irritant and non-irritant whereas those with scores of 4 and above are classified as irritant. These analyses support the validity of the original four-parameter eye irritation QSAR model for neutral organic chemicals. Furthermore, they provide a method for interrelating sets of in vivo data in which the

biological response parameters are expressed in quite different formats, providing a means of utilizing historical data and thereby extending the availability of in vivo data suitable for the validation of in vitro alternative methods.

Bradlaw JA, Wilcox NL. **Executive Summary: Workshop on eye irritation testing: practical applications on non-whole animal alternatives (Baltimore, November 14-19).** Food Chemical Toxicol 1997;35(1):1-11.
BIOSIS COPYRIGHT: BIOL ABS. In November 1993, over 200 people from 14 nations participated in an IRAG workshop on eye irritation testing. The goal of the workshop was to set a course for the scientific approval and acceptance of non-whole animal alternatives to the Draize eye test. Through a retrospective review of existing in vitro and in vivo data by expert working groups, the endeavour examined the current status of practical application of in vitro alternatives used to predict eye irritation. Over 74 data sets from 59 laboratories were reviewed for approximately 26 different test methods. The submissions were illustrative of varied approaches and broad applications of in vitro assays. It was concluded that: (1) data are insufficient to support the total replacement of in vivo ocular irritancy testing with in vitro methods; (2) alternative methods to the in vivo standard (Draize) are currently being used extensively by industry as screens in the risk assessment process for product development; and (3) based on current practices for ocular irritancy testing, it appears that some models exist that may have the potential to reduce the need for new animal testing, provided they are validated and conducted under well defined conditions. Several recommendations are proffered to facilitate scientific review of in vitro and in vivo data sets in parallel: for example, basic research is encouraged to further identify mechanistic endpoints targeting human ocular injury; a chemical test bank is necessary for testing standards; an international data bank should be established; test batteries need to be identified that adequately test novel substances for safety; a third party needs to take the lead to identify promising tests and to facilitate validation studies; and international harmonization of method development, validation and acceptance should be given high priority. Grateful appreciation is conveyed to the many contributors to this complex endeavour.

Brantom PG, Bruner LH, Chamberlain M, De Silva O, Dupuis J, Earl LK, Lovell DP, Pape WJ, Uttley M, et al. **A summary report of the COLIPA international validation study on alternatives to the Draize rabbit eye irritation test**. Toxicol In Vitro 1997;11(1/2):141-79.
CBAC COPYRIGHT: CHEM ABS The principal goal of this study was to det. whether the results from a set of selected currently available alternative methods as used by cosmetics companies are valid for predicting the eye irritation potential of cosmetics formulations and ingredients and, as a consequence, could be valid replacements for the Draize eye irritation test. For the first time in a validation study, prediction models (PMs) that convert the in vitro data from an assay to a prediction of eye irritation were developed for each alternative method before the study began. The PM is an unequivocal description of the relationship between the in vitro and the in vivo data and allows an objective assessment of the reliability and relevance of the alternative methods. In this study, 10 alternative methods were evaluated using 55 test substances selected as representative of substances commonly used in the cosmetics industry (23 ingredients and 32 formulations). Twenty of the single ingredients were common to the European Commission/British Home Office (EC/HO) eye irritation validation study (Balls et al., 1995b). The test substances were coded and supplied to the participating labs. The results were collected

centrally and analyzed independently, using statistical methods that had been agreed before the testing phase began. Each alternative method was then evaluated for reliability and relevance in assessing eye irritation potential. Using the criteria of both reliability and relevance as defined in the study, the preliminary results indicate that none of the alternative methods evaluated could be confirmed as a valid replacement for the Draize eye irritation test across the full irritation scale. However, three alternative methods-the fluorescein leakage test, the red blood cell assay (classification model) and the tissue equiv. assay-each satisfied one criterion of reliability or relevance. Further investigation of the decoded data from this study to explore more fully the relationship between the in vitro data and the in vivo data is recommended. Such a review may allow the development of new prediction models to be tested in a subsequent validation study.

Cristovao AJ, Capela AN, Carvalho CM. **Ca2+ stores in the chick embryo retina cells.** Cell Signal 1997;9(1):97-103.
The Ca2+ stores of digitonin permeabilized chick embryo retina cells in culture were characterized, by using the fluorescence of Fluo-3 potassium salt to follow continuously the free [Ca2+] in the medium. After ATP dependent Ca2+ accumulation, the Ca2+ release was induced by several agents; 10 microM cyclic-ADP-ribose (cADPR), 40 microM Ins (1,4,5)P3 10 microM thapsigargin (Th), 25 microM ionomycin (Ion), 15 microM CCCP together with 4.5 micrograms/ml oligomycin (CCCP/Olig), 50 microM arachidonic acid (AA). Neither Ins(1,4,5)P3 nor cADPR were able to mobilize Ca2+ from internal stores in these cells, but Th and AA were effective in releasing Ca2+. Four major Ca2+ stores in chick embryo retina cells were distinguished: i) the thapsigargin sensitive Ca2+ store, most likely the ER; ii) the Ca2+ store sensitive to oligomycin and CCCP, most likely the mitochondrial Ca2+ store, iii) an AA sensitive Ca2+ store, which is distinct from the previous two; and, iv) the Ca2+ store only sensitive to ionomycin. The capacities of these different Ca2+ stores of the chick embryo retina cells, relative to the total intracellular stores, are: 63.3%, 14.1%, 8.2%, for the ER, the mitochondrial and for the AA sensitive Ca2+ stores, respectively.

Doughty MJ. **Changes in lactate dehydrogenase activity in bovine corneal stroma and epithelium in response to in vitro toxic challenges in the enucleated eye test.** Optom Vis Sci 1997;74(4):198-206.

Espersen RJ, Olsen P, Nicolaisen GM, Jensen BL, Rasmussen ES. **Assessment of recovery from ocular irritancy using a human tissue equivalent model.** Toxicol In Vitro 1997;11(1-2):81-8.
BIOSIS COPYRIGHT: BIOL ABS. The SKIN2 ZK1200 tissue equivalent model has been used in an exploratory study of recovery from ocular irritation. Nine substances from the ECETOC eye irritation reference chemicals data bank were tested. The cellular viability of the tissue model was measured using the MTT assay immediately after chemical exposure and after incubation periods corresponding to observation times used in the Draize tests. The cellular viability of the tissue specimens exposed to moderate irritants, with modified maximum average score (MMAS) values between 15 and 40 (n = 5), returned to control levels within 1-7 days. Tissue specimens exposed to 1 and 10% benzalkonium chloride showed significantly reduced MTT activities at day 14 and day 21. Mild ocular irritants, with MMAS values below 3 (n = 2), did not induce significant depressions of the MTT activities of the tissue specimens after 60 min exposure. A relatively good correlation (r = 0.73) was obtained between the exposure times used in vitro and Draize MMAS values. Moreover, the incubation periods needed for the

exposed SKIN2 ZK1200 to regain control MTT activities showed a good agreement (r > 0.90) with days-to-clear.

Gilleron L, Coecke S, Sysmans M, Hansen E, Van Oproy S, Marzin D, Van Cauteren H, Vanparys P. **Evaluation of a modified HET-CAM assay as a screening test for eye irritancy**. Toxicol In Vitro 1996;10(4):431-46.
The ability of a modified hen's egg test/chorioallantoic membrane (HET-CAM) assay to serve as an in-vitro screening test for eye irritancy was evaluated. The purpose of the study was to determine if an improved version of the HET-CAM assay could be used as an alternative to the in-vivo Draize eye irritation test. The conventional HET-CAM assay was modified by using a microscope to observe the endpoints, hemorrhage and lysis of blood vessels and coagulation of albumin occurring during a 5 minute period, in place of visual observation and by utilizing a custom designed test substance applicator (TSA) to eliminate the rinsing required in the original assay. The TSA was made of a double Teflon ring into which a perlon mesh was locked. The TSA also enabled solids and formulations such as creams, lotions, and suspensions to be tested as well as liquids. The modified HET-CAM assay was first tested with three known irritants, benzalkonium-chloride (8001545) (BAC), dimethylformamide (68122) (DMF), and imidazole (288324) representing a surfactant, liquid, and solid, respectively. Testing was then extended to 46 compounds that had been tested previously in the bovine corneal opacity and permeability (BCOP) assay for the European Community (EC). In-vitro eye irritation scores were calculated from the three HET-CAM endpoints for each compound and compared against published results obtained using the Draize test according to the criteria: the maximum average scores (MAS) using thresholds of 15.0 and 25.0, the Kay and Calandra method, and criteria developed by the EC. BAC, DMF, and imidazole induced dose related responses in the HET-CAM assay. When applied to the 46 compounds in the BCOP/EC dataset, 19 were classified as nonirritants and 27 as irritants. The correlations between the HET-CAM and Draize test data yielded correlation coefficients of 0.78 to 0.93. The sensitivity, specificity, and predictability of the HET-CAM assay when judged against the most rigorous classification of the Draize test (MAS threshold = 15.0) was 80, 81.3, and 80.4%, respectively. The authors conclude that the modified HET-CAM assay appears to be a promising alternative to the Draize test.

Mathers PH, Grinberg A, Mahon KA, Jamrich M. **The Rx homeobox gene is essential for vertebrate eye development.** Nature 1997;387(6633):603-7.
Development of the vertebrate eye requires a series of steps including specification of the anterior neural plate, evagination of the optic vesicles from the ventral forebrain, and the cellular differentiation of the lens and retina. Homeobox-containing genes, especially the transcription regulator Pax6, play a critical role in vertebrate and invertebrate eye formation. Mutations in Pax6 function result in eye malformations known as Aniridia in humans and Small eye syndrome in mice. The Drosophila homologue of Pax6, eyeless, is also necessary for correct invertebrate eye development, and its misexpression leads to formation of ectopic eyes in Drosophila. Here we show that a conserved vertebrate homeobox gene, Rx, is essential for normal eye development, and that its misexpression has profound effects on eye morphology. Xenopus embryos injected with synthetic Rx RNA develop ectopic retinal tissue and display hyperproliferation in the neuroretina. Mouse embryos carrying a null allele of this gene do not form optic cups and so do not develop eyes. The Rx gene family plays an important role in the

establishment and/or proliferation of retinal progenitor cells.

Spielmann H. **Ocular irritation**. In Vitro Methods Pharm Res 1997;265-87.
CBAC COPYRIGHT: CHEM ABS A review with 42 refs. Use and limitations of the Draize rabbit's eye test, refinements of the Draize test, in vitro alternatives for assessing ocular irritation, and validation of in vitro alterations to Draize test were discussed.

PHARMACOKINETIC AND MECHANISTIC STUDIES

Abbas R, Hayton WL. **A physiologically based pharmacokinetic and pharmacodynamic model for paraoxon in rainbow trout.** Toxicol Appl Pharmacol 1997;145(1):192-201.
Trout were exposed to an aqueous solution of 75 ng/ml paraoxon for 5 days at 12 degrees C. The relationships among paraoxon concentration in water and target organs, AChE inhibition, and carboxylesterase (CaE) detoxification of paraoxon were characterized quantitatively by development of a PBPK-PD model. The PKPD model structure consisted of brain, heart, liver, kidney, and remainder of the body, which were interconnected by blood circulation. The paraoxon tissue/blood partition coefficients were: plasma/water, 1.46; liver/plasma, 5.89; brain/plasma, 3.90; heart/plasma, 2.91; kidney/plasma, 0.45; and blood/plasma, 0.91. Turnover of AChE was characterized from a dose-response study, in which its zero-order synthesis rate and first-order degradation rate constant were determined in several tissues; for brain they were 7.67 pmol/min and 7.31 x 10(-5) hr(-1). The uptake and depuration clearances of paraoxon (Cl(u) = 0.651 and Cl(d) = 0.468 ml min(-1) g body wt(-1)) were determined using a compartmental model. During continuous water exposure to paraoxon, AChE activity in the tissues declined to new steady state values that were maintained by the synthesis of new AChE. CaE was shown by simulation to be an important pathway for detoxification of paraoxon.

Althaus JS, Fici GJ, Plaisted SM, Kezdy FJ, Campbell CM, Hoogerheide JG, Von Voigtlander PF. **Protein nitration by peroxynitrite: a method for monitoring nitric oxide neurotoxicity**. Microchem J 1997;56(2):155-64.

Andersen ME, Eklund CR, Mills JJ, Barton HA, Birnbaum LS. **A multicompartment geometric model of the liver in relation to regional induction of cytochrome P450s.** Toxicol Appl Pharmacol 1997;144 (1):135-44.
BIOSIS COPYRIGHT: BIOL ABS. A geometric, multicompartment model of the liver was developed to examine regional protein induction and to provide model output suitable for predicting the degree of induction in both the whole liver and in specific regions. The model was based on functional hexagonal arrays within the liver. A geometric representation was used to divide these functional units into five zones: a concentric periportal zone, a fenestrated periportal region that interconnects among multiple functional units, and three concentric centrilobular areas, referred to, respectively, as zones 1 through 5. The surface areas (and volumes for hexagonal cylinders) of these five zones were, respectively, 13.5, 25.2, 33.9, 20.3, and 6.8% of the total liver. The pharmacokinetic model for induction had dissociation constants (Kd) and Hill constants (n) for interactions of transcriptional activator-ligand complexes with response elements on DNA. Estimates of regional induction were converted to color intensities to paint

the two-dimensional liver for a visual comparison with immunohistochemical observations. To obtain sharp moving boundaries of induced areas with increasing dose (as noted in various experiments), n values in each subcompartment must be large. To create realistic total induction curves that are relatively smooth, the differences in Kd values between adjacent subcompartments must be less than fivefold. Because of the high n values, the low-dose induction characteristics predicted with the multicompartment liver model differ significantly from those predicted with a model that considers the liver as a single homogeneous compartment.

Andreopoulos D, Kasi LP. **99mTc-labeled diphytanoylphosphatidylcholine liposomes: in vitro and in vivo studies**. J Microencapsul 1997;14(4):427-36.
CBAC COPYRIGHT: CHEM ABS The uniquely structured diphytanoylphosphatidylcholine (DphPC) forms liposomes more stable than conventional straight chain phospholipids. In this study DphPC and pegylated DphPC (DphPC-PEG) liposomes were radiolabeled and evaluated in vitro and in vivo. 99mTc-DphPC liposomes were found to be nontoxic to human white blood cells in vitro. In addn. 99mTc labeled DphPC-PEG liposomes were evaluated as a nonspecific infection imaging agent in a mouse model. Infection sites were imaged within 30 min postinjection, and the radiopharmaceutical exhibited a remarkable in vivo stability. As their biodistribution and pharmacokinetic patterns can be size-modulated, DphPC-based liposomes are excellent candidates for diagnostic and therapeutic applications.

Barton P, Davis AM, McCarthy DJ, Webborn P. **Drug-phospholipid interactions. 2. Predicting the sites of drug distribution using n-octanol/water and membrane/water distribution coefficients**. J Pharm Sci 1997;86(9):1034-9.
CBAC COPYRIGHT: CHEM ABS The in vivo tissue distribution of seventeen drugs has been modeled by using estd. n-octanol/water and membrane/water distribution coeffs. In this study, the membrane affinities are estd. using the new technique of immobilized artificial membrane (IAM) column chromatog. DELTA(Log D(n-octanol/water-membrane/water)), which measures a hypothetical equil. of the drug between of n-octanol and membrane phase, is a better model of in vivo tissue distribution, as measured by Adipose Tissue Storage Index (ASI), than either n-octanol/water or membrane/water distribution coeffs. alone. This demonstrates the importance of membrane distribution coeffs. as a complementary descriptor of lipophilicity to n-octanol/water distribution coeffs., in modeling in vivo distribution of drugs. This rapid method for predicting in vivo distribution of drugs, based on n-octanol and membrane/water distribution coeffs., may be a useful tool to aid to the selection of drugs with beneficial pharmacokinetic profiles.

Bogdanov MR, Migranov MG. **[Mechanism of action of neurotoxic insecticides]**. Agrokhimiya 1996; (12):110-21. (Rus)
BIOSIS COPYRIGHT: BIOL ABS. RRM LITERATURE REVIEW TOXICOLOGY PESTICIDES ACTION MECHANISM NEUROTOXIC INSECTICIDES STRUCTURAL DIVERSITY CLASSIFICATION NEUROTOXIC INSECTICIDE RECEPTORS STRUCTURE NEUROTRANSMITTER RECEPTORS FUNCTION RECEPTOR-TOXIN INTERACTION INSECTICIDE RESISTANCE DDT INSECTICIDE RECEPTOR MODEL ACETYL CHOLINESTERASE GAMMA-AMINOBUTYRIC ACID RECEPTOR BIOCHEMISTRY AND BIOPHYSICS.

Bonate PL, Swann A, Silverman PB. **Preliminary physiologically based pharmacokinetic model for cocaine in the rat: model development and scale-up to humans**. J Pharm Sci 1996 Aug; 85: 878-83.
IPA COPYRIGHT: ASHP A physiologically based multicompartmental pharmacokinetic model for cocaine was developed using the rat as a prototype species, and the model was scaled up to humans using previously reported data for 16 male subjects who received 20.5 mg cocaine by intravenous (IV) injection, 94.3 mg by intranasal administration, or 39.5 mg by smoke inhalation. Compartments included in the model were brain, heart, gut, liver, muscle, fat, venous blood, arterial blood, and a mass balance compartment. Drug delivery to the tissues was assumed to be flow limited. The model incorporated a nonsaturable binding site for cocaine in the liver. Elimination occurred via both blood and hepatic elimination. The model accurately predicted cocaine levels in humans after intranasal and inhalation administration. However, a poor fit was observed after IV injection.

Botsman K, Tickle K, Smith JD. **A Bayesian formulation of the Kalman filter applied to the estimation of individual pharmacokinetic parameters**. Comput Biomed Res 1997;30(2):83-94.

Boyd GW, Coombs MM, Ioannides C, Lewis DF, Snelling J, Tsakalof A. **Species variation in the metabolism of 15,16-dihydro-11-methylcyclopenta(a)phenanthren-17-one to its 3,4-dihydrodiol, the proximate carcinogen**. Carcinogenesis 1995;16(10):2351-5.
A comparison study was conducted on the in-vitro metabolism of 15,16-dihydro-11-methylcyclopenta(a) phenanthren-17-one (5836851) (15,16-DMP-17-one) in mammalian liver microsomal preparations and that of its metabolite, 3,4-dihydrodiol-11-methyl-3,4,15,16-tetrahydrocyclopenta(a)phenan- thren-17-one (3,4-dihydrodiol). Liver samples were obtained from golden-Syrian-hamsters, Wistar-albino-rats, BALB/c-mice, New-Zealand-rabbits, a beagle-dog, cynomolgus-monkeys, and man for the preparation of liver microsomal fractions. Carbon-14 labeled 15,16-DMP-17-one was incubated with the microsomal fractions, followed by high pressure liquid chromatography and mass spectrometry for the separation and analysis of the parent compound and its metabolites. In all animals, metabolic activation was observed at ring-A, a five membered ring-D, and at the 11-methyl group to produce the same range of metabolites, although there was substantial variation in the amounts of individual metabolites. The production of 3,4-dihydrodiol was observed in all animal species, with the implication that all the species studied may be susceptible to the carcinogenic effects of this metabolite, although the effects have only been noted in rat and mouse studies. The authors suggest that the distribution of metabolites may depend on the active site of cytochrome-P450-1A1.

Breimer DD. **Integrated pharmacokinetic and pharmacodynamic approach to controlled drug delivery**. J Drug Target 1996;3(6):411-5.
IPA COPYRIGHT: ASHP The assessment of an optimal controlled drug delivery regimen that requires integrated pharmacokinetic/pharmacodynamic/clinical research strategies, in which both rate and time control are important issues, is examined. Pharmacokinetic/pharmacodynamic modeling, time dependence, and rate dependence are discussed.

Brown RP, Delp MD, Lindstedt SL, Rhomberg LR, Beliles RP. **Physiological parameter values for physiologically based pharmacokinetic models**. Toxicol Ind Health 1997;13(4):407-84.

Buckheit RJr, Hollingshead M, Stinson S, Fliakas-Boltz V, Pallansch LA, Roberson J, Decker W, Elder C, Borgel S, et al. **Efficacy, pharmacokinetics, and in vivo antiviral activity of UC781, a highly potent, orally bioavailable non-nucleoside reverse transcriptase inhibitor of HIV type 1**. Aids Res Hum Retroviruses 1997;13(9):789-96.
CBAC COPYRIGHT: CHEM ABS A series of compds. related to oxathiin carboxanilide has been identified as non-nucleoside reverse transcriptase inhibitors (NNRTIs) of HIV-1, and structure-activity relationships have been described (Buckheit RW, et al.: Antimicrob Agents Chemother 1995;39:2718-2727). Three new analogs (UC10, UC82, and UC781) inhibited lab. and clin. isolates of HIV-1, including isolates representative of the various clades of HIV-1 found worldwide, in both established and fresh human cells. Virus isolates with the amino acid changes L100I, K103N, V106I, and Y181C in the reverse transcriptase were partially resistant to these compds. However, UC781 inhibited these virus isolates at low nontoxic concns., presenting a broad in vitro therapeutic index. As with other NNRTIs, each of the compds. synergistically interacted with AZT to inhibit HIV-1 replication. UC781 possesses a favorable pharmacokinetic profile in mice with a high level of oral bioavailability. Plasma concns. reached max. levels within 2 to 4 h of oral administration and remained in excess of those required for in vitro anti-HIV activity for at least 24 h after a single oral dose. When evaluated in a murine hollow fiber implant model of HIV infection, UC781 dosed orally or parenterally was able to suppress HIV replication completely in this model system, providing evidence of the in vivo efficacy of the compd.

Bwijo B, Alin MH, Abbas N, Wernsdorfer W, Bjorkman A. **Efficacy of artemisinin and mefloquine combinations against Plasmodium falciparum. In vitro simulation of in vivo pharmacokinetics.** Trop Med Int Health 1997;2(5):461-7.

Chambers JE, Carr RL. **Biochemical mechanisms contributing to species differences in insecticidal toxicity**. Toxicology 1995;105(2-3):291-304.
Biochemical mechanisms and their possible contribution to observed interspecies differences in insecticidal toxicity were discussed. The general classes of insecticides were described. These include chlorinated hydrocarbon insecticides, cyclodienes, anticholinesterase insecticides, and synthetic pyrethroid insecticides. The class of anticholinesterase insecticides includes organophosphorus and carbamate insecticides, which are esters of phosphoric-acid and carbamic-acid, respectively. Factors influencing the toxicity of insecticides to mammals were discussed. Sensitivity of the target site is considered to be an important factor. Disposition and metabolism, however, are also significant factors contributing to insecticide toxicity. Phosphorothionate insecticides, for example, must be activated by cytochrome-P-450 (P450) isozymes to their own oxon (phosphate) metabolite to become a potent anticholinesterase agent. All classes of insecticides, although they exert different types of molecular action within the nervous system, cause the same general neurotoxic effect, hyperexcitability. Species differences in insecticide toxicity were discussed. The discussion included a summary of the median lethal dose (LD50) and concentration (LC50) concept. Considerable variation in the LD50s and LC50s exist across various species. LD50s ranging from 50 to 250 to more than 10,000mg/kg have been measured in birds and mammals. LC50s varying from below 10 to more than 10,000 micrograms per liter have been measured in fish species. Among the anticholinesterase agents, organophosphates appear to be the most toxic to mammals, followed by phosphorothionates and carbamates. In birds,

organophosphates and carbamates have been shown to be highly toxic followed by the phosphorothionates. Pyrethroids generally have low toxicity to mammals and birds. Factors found to affect phosphorothionate toxicity in rats were discussed. These include acetylcholinesterase sensitivity.

Chen C, Pollack GM. **Extensive biliary excretion of the model opioid peptide [D-PEN2,5] enkephalin in rats**. Pharm Res 1997;14(3):345-50.
CBAC COPYRIGHT: CHEM ABS This study was designed to test the hypothesis that the enzymically stable opioid peptide, [D-Pen2,5]-enkephalin (DPDPE), is excreted extensively into bile. Following an i. v. bolus dose of DPDPE (10 mg/kg) to rats, concns. of DPDPE in serum, bile, liver homogenate and urine were measured by a novel capillary zone electrophoresis method. Data were analyzed to recover the fundamental pharmacokinetic parameters (vols. of distribution; distribution and elimination rate consts. governing DPDPE systemic and biliary disposition). Parallel in vitro expts. were performed to evaluate the partitioning of DPDPE between erythrocytes and plasma, as well as to assess the degree of binding of DPDPE to serum proteins. The majority of the administered dose (.apprx.80%) was recovered from bile as intact peptide. DPDPE disposition was best described by a two-compartment model with Michaelis-Menten elimination (Km: 37.5 mug/mL; Vmax: 1143 mug/min/kg) from the central compartment into bile, suggestive of an active hepatic transport system. DPDPE was assocd. with a distributional space of 486 mL/kg. In vitro incubation of DPDPE with whole blood showed that . apprx.65% of the peptide was assocd. with erythrocytes. The difference between concns. of DPDPE in erythrocytes and plasma was statistically significant (29.2 vs. 18.1 mug/mL), but not between whole blood and plasma (21.3 vs. 18.1 mug/mL). Concn.-independent binding of DPDPE to serum proteins was evidenced between 10 and 100 mug/mL, with an unbound fraction of 0.517. DPDPE undergoes extensive biliary excretion after i.v administration in rats. The apparent nonlinearity in the biliary excretion of DPDPE revealed by the pharmacokinetic modeling strongly suggests the existence of an active transport system(s) in hepatocytes which may mediate the rapid disappearance of DPDPE from the systemic circulation.

Chen S, Qian M, Brenna J, Gallo JM. **Determination of antisense phosphorothioate oligonucleotides and catabolites in biological fluids and tissue extracts using anion-exchange high-performance liquid chromatography and capillary gel electrophoresis**. J Chromatogr B Biomed Appl 1997;692 (1):43-51.
CBAC COPYRIGHT: CHEM ABS Chem. modified phosphorothioate oligodeoxynucleotides (ODNs) have become crit. tools for research in the fields of gene expression and exptl. therapeutics. Bioanal. assays were developed that utilized fast anion-exchange high-performance liq. chromatog. (HPLC) and capillary gel electrophoresis (CGE) for the detn. of 20-mer ODNs in biol. fluids (plasma and urine) and tissues. A 20 mer ODN in the antisense orientation directed against DNA methyltransferase (denoted as MT-AS) was studied as the model ODN. The anion-exchange HPLC method employed a short column packed with non-porous polymer support and a ternary gradient elution with 2 M lithium bromide contg. 30 formamide. Anal. of the MT-AS is accomplished within 5 min with a detection limit of approx. 3 ng on-column at 267 nm. For plasma and urine, samples were dild. with Nonidet P-40 in 0.9 NaCl and directly injected onto the column, resulting in 100 recovery. For tissue homogenates, a protein kinase K digestion and phenol-chloroform extn. were used, with an av. recovery of about 50. Since the HPLC assay cannot provide one-base sepn., biol. samples were also processed by an anion-exchange solid-

phase extn. and a CGE method to characterize MT-AS and its catabolites of 15-20-mer, species most relevant to biol. activity. One base sepn., under an elec. field of 400 V/cm at room temp., was achieved for a mixt. of 15-20-mer with about 50 pg injected. Assay validation studies revealed that the combined HPLC-CGE methods are accurate, reproducible and specific for the detn. of MT-AS and its catabolites in biol. fluids and tissue homogenates, and can be used for the pharmacokinetic characterization of MT-AS.

Chen Z, Liu J, Zhao Z. **[Generalized stochastic compartmental pharmacokinetic model based on Markov process]**. Shandong Daxue Xuebao, Ziran Kexueban 1997;32(1):31-4. (Chi)
CBAC COPYRIGHT: CHEM ABS A class of generalized stochastic compartmental models based on Markov processes are considered. Some stochastic formulations are established by using the basic data which is obtained by the injection of 47Ca in plasma.

Copley SD. **Diverse mechanistic approaches to difficult chemical transformations microbial dehalogenation of chlorinated aromatic compounds**. Chem Biol 1997;4(3):169-74.
BIOSIS COPYRIGHT: BIOL ABS. RRM JOURNAL ARTICLE DIFFICULT CHEMICAL TRANSFORMATIONS CHLORINATED AROMATIC COMPOUNDS POLLUTANTS MICROBIAL DEHALOGENATION BIODEGRADATION MICROBIAL DEHALOGENASES DIVERSE MECHANISTIC APPROACHES ENZYMOLOGY TOXICOLOGY.

Cruz T, Gaspar R, Donato A, Lopes C. **Interaction between polyalkylcyanoacrylate nanoparticles and peritoneal macrophages: MTT metabolism, NBT reduction, and NO production**. Pharm Res 1997 Jan;14:73-9.
IPA COPYRIGHT: ASHP The in vitro toxicity of various polyalkylcyanoacrylate (PACA) nanoparticles on resident and thioglycolate-elicited peritoneal macrophages isolated from mice was investigated, and cellular viability, oxidative burst, and nitric oxide (NO) production were assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, nitroblue tetrazolium (NBT) reduction, and nitrite evaluation, respectively. Polymethylcyanoacrylate (PMCA), polyethylcyanoacrylate (PECA), poly(butyl cyanoacrylate) (polybutylcyanoacrylate; PBCA), and polyisobutylcyanoacrylate (PIBCA) nanoparticles were studied. PMCA and PBCA nanoparticles and lipopolysaccharide (LPS) were used to assess microphage nitrite production. PACA nanoparticles produced cellular morphological modifications and induced toxicity in both types of macrophages. The uptake of PACA nanoparticles by the macrophages caused an increase in respiratory burst and induced the release.

Dedik L, Durisova M. **General moments in linear pharmacokinetic models**. Clin Res Regul Aff 1996;13(3-4):199-210.
IPA COPYRIGHT: ASHP The derivation and application of formulas for estimating the parameters of mean residence time (MRT) and variance of residence of the drug in the body (VRT) of the linear or linearized complex time invariant pharmacokinetic systems, with or without shunt and time delays, defined on the animal body after any route of drug administration are discussed; the derivation is based on the model of the system transfer function.

Dedik L, Durisova M. **New general formulas for estimation of mean residence time and its variance**. Pharmazie 1997;52(5):404-5.
CBAC COPYRIGHT: CHEM ABS General formulas are given to est. system parameters MRT(mean residence time) and its VRT(variation residence time) in pharmacokinetic system models after oral administration of drug veralipride as a soln.

Fagiolino P, Eiraldi R, Vazquez M. **[Flow-dependent pharmacokinetic model. Clinical application to digoxin]**. Acta Farm Bonaerense 1996;15(4):225-38. (Spa)
CBAC COPYRIGHT: CHEM ABS A compartmental pharmacokinetic model (SIMULFIS) which considers the body fluid systems is proposed. The effects of varying cardiac output and organ blood flows on digoxin pharmacokinetics and plasma and tissue levels were studied. Digoxin-amiodarone interaction was analyzed according to this model. Simulated digoxin levels showed an increased tissue uptake and a reduced plasma concn. of drug during phys. exercise. The SIMULFIS model assumes a single mechanism for the digoxin-amiodarone interaction, which explains the increased oral bioavailability, the reduced hepatic and renal elimination, and the unaltered distribution vol. of digoxin.

Geyer HJ, Schramm KW, Scheunert I, Schughart K, Buters J, Wurst W, Greim H, Kluge R, Steinberg CE, Kettrup A, et al. **Considerations on genetic and environmental factors that contribute to resistance or sensitivity of mammals including humans to toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds. Part 1. Genetic factors affecting the toxicity of TCDD.** Ecotoxicol Environ Saf 1997;36(3):213-30.
The marked species differences in short-term toxicity (30-day LD50) of ca. 10,000 (LD50: guinea pigs ca. 1 microgram/kg body wt and Han/Wistar Kuopio rats more than 9600 micrograms/kg body wt) of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is one of the central issues of the controversies that have developed on the validity of risk assessment strategies for TCDD and related compounds. One of the most challenging issues that toxicologists face today is the identification of genes that contribute to or are responsible for increased resistance or sensitivity to TCDD and related compounds. It is assumed that most, if not all, toxic effects of TCDD are mediated more or less through the binding affinity to the Ah receptor. This hypothesis was extended and tries to explain the differences in sensitivity/resistance of animals including humans to TCDD by their total fat (lipid) content. In this respect the gene or genes which is or are responsible for obesity of mammals including humans are of great interest. An obvious linear positive logarithmic relationship between the oral 30-day LD50 (microgram/kg) of TCDD in different species and strains of mammals and their total body fat content (TBF%) was found: log LD50 = 5.30 x log (TBF)-3.22, or LD50 = 0.000603 x (TBF)5.30. By means of this regression the toxicity of TCDD in mammals including humans of different age and/or body weight can be predicted if their total body fat content is known. Examples of single-gene and polygenic disease models in different mammals, such as nonobese diabetic, diabetic, viable yellow, obese, and fat mice, as well as transgenic mice, and other suitable animal models, such as fatty Zucker rats, Han/Wistar (Kuopio) rats, and minipigs, are discussed, and predicted LD50 values of TCDD in these animals and humans are presented.

Herault JP, Donat F, Barzu T, Crepon B, Bernat A, Lormeau JC, Herbert JM. **Pharmacokinetic study of three synthetic AT-binding pentasaccharides in various animal species-extrapolation to**

humans. Blood Coagul Fibrinol 1997;8(3):161-7.

Hossain M, Wright E, Baweja R, Ludden T, Miller R. **Nonlinear mixed effects modeling of single dose and multiple dose data for an immediate release (IR) and a controlled release (CR) dosage form of alprazolam**. Pharm Res 1997;14(3):309-15.
CBAC COPYRIGHT: CHEM ABS NONMEM was applied to single dose and multiple dose bioavailability data for an IR and a CR dosage form of alprazolam to acquire addnl. information from the data which are not easily obtainable by traditional means. The objective function value (OBJ) and diagnostic plots were used as measures of goodness of fit of the model to the data. A change in the OBJ value of 7.9 was necessary to show statistical significance between 2 models when the 2 models differed by 1 parameter. A 2-compartment linear model with first-order absorption and elimination best describes the data. Including a lag time, 2 different rates of absorption (KAIR and KACR), and bioavailability for the CR relative to the IR dosage form significantly improved the fit of the model to the data. Cigaret smoking was assocd. with a 100% increase in clearance of alprazolam as compared to non-smokers. The higher residual variability obsd. in this study, where interoccasion variability (IOV) was not initially modeled, could be explained to a large extent by the presence of significant interoccasion variability (IOV). Since alprazolam has been suggested to be mainly metabolized by the CYP3A4 isoenzyme in humans, it appears that tobacco could be an inducer of CYP3A4 and/or alprazolam may be metabolized by other isoenzyme(s) (specifically, CYP1A1/1A2) that are induced by cigaret smoke. The population pharmacokinetic model approach combined with exploratory graphical data anal. is capable of identifying important covariates from well-controlled data rich Phase I studies early in drug development.

Jarugula VR, Lam SS, Boudinot FD. **Nonlinear pharmacokinetics of 5-fluorouracil in rats.** J Pharm Sci 1997;86(6):756-8.
The effects of dose on the pharmacokinetics of 5-fluorouracil (5-FU) were investigated following intravenous administration of 5-FU at 10, 50, and 100 mg/kg to adult male Sprague-Dawley rats. Six rats were studied at each dose level. The dose-normalized area under the curve (AUC) was significantly higher after administration of 100 mg/kg (1.14 +/- 0.55 mg.h/L/mg; mean +/- SD) than after 50 mg/kg (0.50 +/- 0.18 mg.h/L/mg) or 10 mg/kg (0.43 +/- 0.11 mg.h/L/mg), indicating nonlinear elimination of 5-FU in rats. Dose- and time-average pharmacokinetic parameters were calculated by area/moment analysis. The systemic clearance of 5-FU following administration of 100 mg/kg was significantly lower (1.1 +/- 0.49 L/h/kg) than after 50 mg/kg (2.2 +/- 0.72 L/h/kg) or 10 mg/kg (2.4 +/- 0.67 L/h/kg). There was no significant difference in renal clearance values between the three doses (0.47 +/- 0.26 L/h/kg). However, nonrenal clearance was significantly lower after the 100-mg/kg dose (0.77 +/- 0.2 L/h/kg) than after the 50-mg/kg (1.65 +/- 0.49 L/h/kg) and 10-mg/kg (1.87 +/- 0.75 L/h/kg) doses. There was no significant difference between the steady-state volume of distribution values (0.91 +/- 0.36 L/kg) at the three doses. The lower nonrenal clearance following the 100-mg/kg dose compared with that after the lower doses of 5-FU suggested nonlinear metabolism of 5-FU in rats. A two-compartment pharmacokinetic model with parallel first-order (renal excretion) and Michaelis-Menten elimination from the central compartment was simultaneously fit to mean plasma 5-FU concentration versus time data for the three doses. The maximum volume (Vmax) and Michaelis constant (Km) values averaged (mean +/- SE) 8.3 +/- 2.3 mg/h and 31.6 +/- 11.9 mg/L, respectively. The information obtained in this

study will be valuable for the evaluation of prodrugs of 5-FU that are designed to reduce toxicities and to improve oral bioavailability of the anticancer agent.

Johnson AP, Fairman MP. **The identification and purification of a novel mammalian DNA ligase.** Mutat Res 1997;383(3):205-12.
Using a combination of biochemical fractionation and adenylation assays, we have purified a novel 44 kDa protein from human cells which rejoins DNA double-strand breaks. Its rejoining properties and its ability to form an adenylation product with ATP, which can be rapidly dissociated by the presence of DNA breaks, show that this protein is a DNA ligase. As four mammalian DNA ligases have been previously identified we have named this DNA ligase V. Silver staining of the most purified fraction on denaturing polyacrylamide gels reveals a protein doublet of 46/44 kDa of which only the lower band becomes adenylated. Assay of this protein, along with two defined DNA ligases, against DNA templates containing either double and single-strand breaks shows that unlike other DNA ligases, DNA ligase V does not join nicked templates with high efficiency. However, this DNA ligase can join double-strand breaks with a similar efficiency to DNA ligase 1. This result indicates that there may be different types of DNA ligases in mammalian cells which may have specific cellular functions.

Jongeneelen FJ. **Methods for routine biological monitoring of carcinogenic PAH-mixtures.** Sci Total Environ 1997;199(1-2):141-9.
The ability of a biomarker to provide an assessment of the integrated individual dose following uptake through multiple routes is especially valuable for mixtures of polycyclic aromatic hydrocarbons (PAH), due to methodological and practical difficulties of collecting and analysing samples from the various environmental compartments like air, water and soil and various media such as diet, cigarette smoke and workroom air. Since 1980, a large variety of novel approaches and techniques have been suggested and tested, e.g. urinary thioethers, mutagenicity in urine, levels of PAH or PAH-metabolites in blood and urine and methods for determination of adducts in DNA and proteins. Two approaches are more frequently reported: PAH-DNA-adduct monitoring in blood cells and urinary 1-hydroxypyrene monitoring. A large research effort has been made to use the extent of binding of PAH to DNA as a biomarker of exposure. The 32P-post-labeling assay detects the total of aromatic DNA-adducts and the adduct level in white blood cells is claimed to be an indicator of the biological effect of the PAH-mixture. However, the levels of aromatic DNA-adducts may be subject to appreciable analytical and biological variation. The present technical complexity of the method makes it more convenient for research applications than for routine application in occupational health practice. Pyrene is a dominant compound in the PAH mixture and is mainly metabolised to the intermediary 1-hydroxypyrene to form 1-hydroxypyrene-glucuronide, which is excreted in urine. Since the introduction of the determination of 1-hydroxypyrene in urine as a biomarker for human exposure assessment in 1985, many reports from different countries from Europe, Asia and America confirmed the potential of this novel approach. The conclusion of the first international workshop on 1-hydroxypyrene in 1993 was that urinary 1-hydroxypyrene is a solid biological exposure indicator of PAH. Studies with a comparison of several biomarkers confirmed that 1-hydroxypyrene in urine is a valid and sensitive indicator of exposure. Periodical monitoring of 1-hydroxypyrene appears to be a powerful method in controlling occupational PAH-exposure in industries. The reference level and the biological exposure limit of 1-hydroxypyrene in urine are discussed.

Kaina B, Haas S, Kappes H. **A general role for c-Fos in cellular protection against DNA-damaging carcinogens and cytostatic drugs.** Cancer Res 1997;57(13):2721-31.
One of the earliest responses of cells upon exposure to DNA-damaging agents is the induction of c-fos. To elucidate the biological role of Fos expression, we analyzed cells deficient in c-Fos upon treatment with different DNA-damaging agents, including carcinogens and antineoplastic drugs. We show that cells lacking c-Fos are hypersensitive with regard to reproductive cell death, apoptosis, and chromosomal breakage after treatment with agents inducing methylation lesions, bulky adducts, or crosslinks in DNA. They were not significantly hypersensitive to ionizing radiation. The activities of various repair enzymes and glutathione S-transferase and the level of proliferating cell nuclear antigen were not altered in c-fos-/- fibroblasts. Furthermore, the cells were able to remove the main methylation lesions from DNA. c-Fos-deficient cells exhibited a more severe mutagen-induced block to DNA replication and were compromised in the abolition of replication blockage. The data provide compelling evidence that c-Fos/activator protein-1 plays a decisive and general role in cellular defense against genotoxic agents, which require DNA replication to induce chromosomal instability. They are consistent with the hypothesis that impaired recovery from DNA replication inhibition upon mutagen exposure is causally involved in c-fos-/- hypersensitivity.

Kakkar P, Jaffery F, Viswanathan PN. **Specific molecular probes for mechanistic studies in toxicology and molecular epidemiology for risk assessment**. J Environ Sci Health C 1996:14(2):105-37.
Molecular probes and their use in mechanistic toxicological and molecular epidemiological studies were reviewed. The evolution, scope, and future prospects of molecular toxicology were discussed using lead (7439921) poisoning as an example. Over the years diagnosing lead poisoning has evolved from being based on taking a history and clinical observations to estimating effects on hemoglobin and assessing morphological changes and subcellular inclusions. Analytical methods for determining lead have shifted from simple spectrophotometry to sophisticated spectrographic methods such as atomic absorption spectrometry, directly coupled plasma spectrometry, neutron activation analysis, and energy dispersive X-ray analysis. Biochemical analyses now center around detecting changes in protein-kinase-C isoforms, nitric-oxide-synthase, or interleukin-4 instead of just delta-aminolevulinic-acid-dehydratase, porphyrins, or unspecified protein markers. Biomarkers that can be used to assess exposure to environmental chemicals were described. Biochemical screening tests that can be used for detecting molecular lesions were discussed. These include assays that can detect DNA damage and changes in p53-protein mediated growth regulation that are associated with malignancies, cytogenetic markers such as micronucleus induction in lymphocytes which are indicative of exposure to mutagens and carcinogens, and urine markers such as desmosine which indicates connective tissue breakdown in pulmonary emphysema. Using molecular probes and other biochemical tests to elucidate early events preceding a toxic response following exposure to a toxicant was considered. Applying metabolism tests in molecular toxicology was discussed. Using molecular probes in cancer risk assessments was considered. Uncertainties associated with using existing models for risk assessments were discussed and illustrated by describing problems encountered when using rodents as models for toxicity and carcinogenesis studies. Receptor mediated pathobiology was discussed and illustrated using chlorinated dioxins and estrogens of chemicals that bind to specific receptors. Applying transgenic technology to molecular

toxicology was considered. Molecular markers that can be used for investigating ecotoxicants were described.

Lawrence GS, Gobas FA. **A pharmacokinetic analysis of interspecies extrapolation in dioxin risk assessment.** Chemosphere 1997;35(3):427-52.
This study entails a pharmacokinetic analysis of the relationship between the external dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin, TCDD) and resulting concentrations of TCDD in internal tissues and organs of humans and rodent species. The methodology is based on the development and testing of physiologically based pharmacokinetic models for several rodent species and humans. The results indicate that the relationship between the external dose of TCDD and resulting TCDD concentrations in liver and adipose tissue of humans and various species of rats and mice can vary by as much as 725 fold, illustrating that humans and experimental animals differ considerably in their ability to convert external dosages of dioxin to tissue concentrations. Interspecies scaling factors are reported to express the differences in tissue concentrations of dioxin between mice, rats and humans in response to an equivalent external dose. The significance of these findings for conducting human cancer and ecological risk assessments is discussed. It is recommended that pharmacokinetic differences be considered explicity in risk estimation, while separately recognizing interspecies differences in pharmacodynamics (sensitivity).

Ledirac N, Delescluse C, De Sousa G, Pralavorio M, Lesca P, Amichot M, Berge JB, Rahmani R. **Carbaryl induces CYP1A1 gene expression in HepG2 and HaCaT cells but is not a ligand of the human hepatic Ah receptor.** Toxicol Appl Pharmacol 1997;144(1):177-82.
In spite of increasing numbers of insecticides used in agriculture, there are serious concerns regarding the potential risks of exposure to these agents. Carbaryl is one of the most important carbamate insecticides and has been used for about 30 years to control a wide range of pests. The study was designed to investigate if, among various insecticides currently used in world agriculture, this compound could induce human CYP1A1, an enzyme known to play an important role in the chemical activation of xenobiotics to genotoxic derivatives. Studies on HepG2 and HaCaT cell lines showed that carbaryl is capable of increasing, in a dose-dependent manner, both the ethoxyresorufin rufin-O-dec, O-deethylase activity and the steady-state concentrations of CYP1A1 mRNA, suggesting a transcriptional activation of this gene. When alpha-naphthoflavone, a partial Ah receptor (AhR) antagonist, and 8-methoxypsoralen, which interferes with the binding of activated AhR to the xenobiotic responsive element (XRE), were added to the cultures, CYP1A1 induction was suppressed. However, competitive binding studies using the 9S enriched fraction of human cytosol indicated that carbaryl did not displace [3H]TCDD from AhR. These data, together with the activation of a XRE-directed CAT reporter gene by carbaryl, suggest that induction of CYP1A1 involves the participation of the AhR and the XRE, but is not mediated by a direct carbaryl-receptor interaction. An alternative ligand-independent mechanism should be considered. Therefore, although carbaryl concentration in food is very low, care should be taken because of its possible adverse effects in human health through liver and skin, given the well established toxicological importance of CYP1A1 induction.

Lennernas H, Fager G. **Pharmacodynamics and pharmacokinetics of the HMG-CoA reductase inhibitors. Similarities and differences.** Clin Pharmacokinet 1997;32(5):403-25.

Liebetrau W, Runger TM, Mehling BE, Poot M, Hoehn H. **Mutagenic activity of ambient oxygen and mitomycin C in Fanconi's anaemia cells.** Mutagenesis 1997;12(2):69-77.
Cellular evidence suggests that Fanconi's anaemia (FA) might be a condition of increased oxygen sensitivity. In order to test this hypothesis, a common shuttle vector assay with the plasmid pZ189 was utilized. We transfected intact, circular plasmid into FA and control lymphoblast and fibroblast host cells maintained at 5 and 20% O2 (v/v). In parallel experiments, host cells were exposed to different concentrations of mitomycin C (MMC), a cross-linking agent towards which FA cells are known to be hypersensitive. Baseline mutation frequencies at 20% oxygen were significantly higher in plasmids passaged through FA lymphoblasts or FA fibroblasts in comparison with passage through the corresponding control cells. Lowering the oxygen concentration during the 48 h transfection period to 5% resulted in a significant decrease of mutation frequencies in plasmids passaged through FA cells. Sequence analysis of plasmids recovered from FA lymphoblasts revealed a mutation hot spot (22% of point mutations with G:C to A:T base substitutions) at base 117 of the supF tRNA gene. This hot spot was present only at 20% oxygen. 59% of the base changes at the hot spot and 39% of the changes elsewhere in the supF gene were C to T transitions (the corresponding figures are 0 and 27% at 5% oxygen), the most common type of base change induced by oxygen. The mutation spectrum observed suggests a role for 8-hydroxydeoxyguanosine in G:C to A:T base substitutions: at 20% oxygen, FA cells displayed 4 times as many G:C to T:A transversions than FA cells kept at 5% O2. In MMC treated cells.

Liu WW, Liu CH, Kuo SC. **HPLC assay of N-ethylphenylacetamide in serum and pharmacokinetics in rabbits**. Chin Pharm J 1996;48(2):149-55.
IPA COPYRIGHT: ASHP An HPLC assay is described and used to evaluate blood levels of N-ethylphenylacetamide in rabbit blood after the rabbits received 30 mg/kg of intravenous N-ethylphenylacetamide; pharmacokinetic parameters were also analyzed. The HPLC method proved simple and reliable and the serum time-concentration profile was well described by a 1-compartment model. The half-life, clearance, and AUC for N-ethylphenylacetamide were 0.4 h, 1.87 l/kg/h, and 35 mcg/ml/h, respectively.

Madaras-Kelly KJ, Moody J, Larsson A, Baeker Hovde L, Rotschafer JC. **Characterization of synergy between ofloxacin, ceftazidime, and tobramycin against Pseudomonas aeruginosa.** Chemotherapy 1997;43(2):108-17.
The purposes of this study were to investigate the potential for synergy between ceftazidime, tobramycin, and ofloxacin against two clinical isolates (PSA 9258, and PSA 9263) of Pseudomonas aeruginosa utilizing time-concentration-kill curves. A pharmacodynamic model was used to simulate one-compartment pharmacokinetics for single-, double-, or triple-drug combinations utilizing two different elimination half-lives (T1/2). Each duplicate experiment was conducted for 24 h in cation-supplemented Mueller-Hinton broth. Synergy, indifference, and antagonism were defined as reductions of > or = 2.0, > or = 0 to < or = 2, or < or = 0 in mean log10 CFU/ml (CFU = colony-forming units) in bacterial counts at any time point.

Mansour K. **An approach for determination of chronopharmacokinetic parameters of methotrexate**. Eur J Drug Metab Pharmacokinet 1997;22(1):41-5.

CBAC COPYRIGHT: CHEM ABS In classical pharmacokinetic studies, the organism is represented by 1 or several compartments described by a system of linear differential equations with const. coeffs. A system of linear differential equations can not describe the compartmental model in the presence of chronobiol. variations. The purpose of this study is to calc. the chronopharmacokinetic parameters of methotrexate, which presents this type of variation.

Marionnet AV, Lizard G, Chardonnet Y, Schmitt D. **Comparative evaluation of the antiproliferative effect of cyclosporin A and gamma-interferon on normal and HPV-transformed keratinocytes by cell counting, MTT assay and tritiated thymidine incorporation.** Cell Biol Toxicol 1997;13(2):115-23.
We compared three techniques, the MTT tetrazolium assay, cell counting, and tritiated thymidine ([3H] TdR) incorporation assay to measure the antiproliferative effect of cyclosporin A (CsA) and interferon-gamma (IFN-gamma) on normal human skin keratinocyte cultures (NHK) used at the second passage and human papilomavirus type 16- and 18-transformed cell lines (EK16 and EK18) exposed continuously to the drugs for 3 days. The three techniques showed that under CsA (0.5 and 8 micrograms/ml) and IFN-gamma (5 and 160 U/ml) treatments the cells remained viable and that the growth of keratinocytes was inhibited. For IFN-gamma, the MTT colorimetric assay consistently underestimated its growth inhibitory activity as compared to cell counting or [3H]TdR incorporation, whatever the cells used. For high doses of CsA, MTT and cell counting gave similar percentages, of inhibitory activity whatever the cells; MTT underestimated this activity as compared to [3H]TdR incorporation only in NHK and EK18 cells, whereas similar results were obtained with EK16 cells. In conclusion, this investigations shows that MTT sensitivity differed with the drug and also according to the keratinocyte cultures. The MTT test is clearly not appropriate for study of IFN-gamma treatment whatever the keratinocytes used. Such discrepancies indicate that the MTT test should be done with care on cultures to measure the effects of drugs on cell growth; the growth inhibition should be carefully considered and it would be best if two different methods were used.

Meineke I, Schmidt W, Nottrott M, Schroeder T, Hellige G, Gundert-Remy U. **Modeling of nonlinear pharmacokinetics in sheep after short-term infusion of cardiotoxic doses of imipramine**. Pharmacol Toxicol 1997;80(6):266-71.
CBAC COPYRIGHT: CHEM ABS Imipramine was administered to sheep by i.v. infusion in high doses (450-900 mg) to elicit cardiovascular shock. A cardiac-assist device was then employed to manage the acute overdose situation. The concn.-time course of imipramine and its metabolite demethylimipramine in plasma was measured by HPLC. As an indicator of imipramine's cardiotoxic effect, cardiac output was monitored. The pharmacokinetics were evaluated under these conditions, and the efficiency of a cardiac-assist device, with and without an integrated hemoperfusion unit, in removing drug from the circulation was assessed. The kinetics of imipramine could be described by a 3-compartment body model with concn.-dependent clearance resulting in nonlinear kinetics. The changes in cardiac output with time could be linked to the pharmacokinetic model by a linear relationship. The cardiac-assist device contributed to the overall elimination of imipramine, whereas the hemoperfusion unit had no clin. relevant impact.

Miller DD, De Los Angeles J, Dalton JT, He M, Zhang X, Shams G, Lei L, Patil PN, Feller DR, Hsu FL.

Biological evaluation of a new series of medetomidine analogs and molecular basis for the binding of medetomidine analogs to alpha2-adrenoceptors. Proc Erdec Sci Conf Chem Biol Def Res 1996;1047-52.

Mouton JW, Vinks AA T, Punt NC. **Pharmacokinetic-pharmacodynamic modeling of activity of ceftazidime during continuous and intermittent infusion**. Antimicrob Agents Chemother 1997;41 (4):733-8.
CBAC COPYRIGHT: CHEM ABS We developed and applied pharmacokinetic-pharmacodynamic (PK-PD) models to characterize in vitro bacterial rate of killing as a function of ceftazidime concns. over time. For PK-PD modeling, data obtained during continuous and intermittent infusion of ceftazidime in Pseudomonas aeruginosa killing expts. with an in vitro pharmacokinetic model were used. The basic PK-PD model was a max.-effect model which described the no. of viable bacteria (N) as a function of the growth rate (lambda) and killing rate (epsilon) according to the equation dN/dt = :lambda - epsilon . cntdot. [Cgamma/(EC50gamma + Cgamma)]: .cntdot. N, where gamma is the Hill factor, C is the concn. of antibiotic, and EC50 is the concn. of antibiotic at which 50% of the max. effect is obtained. Next, four different models with increasing complexity were analyzed by using the EDSIM program (MediWare, Groningen, The Netherlands). These models incorporated either an adaptation rate factor and a max. no. of bacteria (Nmax) factor or combinations of the two parameters. In addn., a two population model was evaluated. Model discrimination was by Akaike's information criterion. The exptl. data were best described by the model which included an Nmax term and a rate term for adaptation for a period up to 36 h. The abs. values for maximal growth rate and killing rate in this model were different from those in the original expt., but net growth rates were comparable. It is concluded that the derived models can describe bacterial growth and killing in the presence of antibiotic concns. mimicking human pharmacokinetics. Application of these models will eventually provide us with parameters which can be used for further dosage optimization.

Nakai D, Fuse E, Suzuki H, Inaba M, Sugiyama Y. **Evaluation of the efficiency of targeting of antitumor drugs: simulation analysis based on pharmacokinetic/pharmacodynamic considerations**. J Drug Target 1996;3(6):443-53.
IPA COPYRIGHT: ASHP Antitumor drugs were used as model agents to consider what kind of drug delivery system (DDS) was effective from a pharmacokinetic/pharmacodynamic point of view using simulation analysis to examine the factors governing the cytotoxicity of drugs in tumors based on a hybrid perfusion model. It is suggested that the increase in tumor tissue binding of drug results in an increased unbound drug mean residence time (MRTtu), leading to the increased activity of cell cycle phase specific (type II) drugs. In contrast, the cytotoxic activity of cell cycle phase nonspecific (type I) drugs is unaffected by the alteration in the tissue binding, since the intracellular AUC defined for unbound drugs (AUCtu) is unaffected by the extent of drug binding. It was also found that the symmetrical increase in the permeability-surface area products (PS) for drug influx (PSinf) and efflux (PSeff) across the tumor plasma membrane results in the unaltered and reduced antitumor activity for the type I and type II drugs, respectively, due to the unaltered AUCtu and to the reduced MRTtu. Optimization of antitumor activity can be attained by increasing the tissue binding for type II drugs and by increasing PSinf and/or by decreasing PSeff for type I and type II drugs.

Niazi SK, Alam SM, Ahmad SI. **Partial-area method in bioequivalence assessment: naproxen**. Biopharm Drug Dispos 1997;18(2):103-16.

Ouellet DM, Pollack GM. **Pharmacodynamics and tolerance development during multiple intravenous bolus morphine administration in rats**. J Pharmacol Exp Ther 1997;281(2):713-20.

Pape WJ. **Validation of in vitro methods to single out photoirritants using mechanistically based tests.** Arch Toxicol Suppl 1997;19239-47.
In the recent OECD draft proposal for a new guideline on acute dermal photoirritation testing, in vitro screening tests have been included as part of the sequential test strategy. These screening tests were placed directly prior to animal tests proposed. In Europe some in vitro techniques - cell culture and mechanistic tests - are under validation in a joint project of the European Center for the Validation of Alternative Methods (ECVAM) and the European Cosmetic, Toiletry, and Perfumery Association (COLIPA). Two promising cellular in vitro tests are presented and discussed as tool for the screening of photoirritancy. The first one as a general core test performed in each participating laboratory is the 3T3 mouse fibroblast Neutral Red Uptake Phototoxicity test determining the cell viability by uptake of Neutral Red as end point, whereas the second performed only by three participating laboratories was the the Red Blood Cell phototoxicity test comprising a combination of two end points, the photohaemolysis and the oxyhaemoglobin oxidation. Besides this, other mechanistic tests can be used as additional support. Identification of photoirritation is generally considered to be one area for the successful research and validation of in vitro techniques.

Parham FM, Kohn MC, Matthews HB, Derosa C, Portier CJ. **Using structural information to create physiologically based pharmacokinetic models for all polychlorinated biphenyls.** Toxicol Appl Pharmacol 1997;144(2):340-7.
Physiologically based pharmacokinetic (PBPK) models are useful in describing the distribution, metabolism, and fate of xenobiotics across multiple species. The eventual goal of the present research is to create PBPK models for all 209 polychlorinated biphenyls (PCBs). Key parameters in any PBPK model are the tissue-to-blood partition coefficients. Tissue:blood partition coefficients relate the compound's concentration in a target tissue to its concentration in blood under equilibrium conditions. Data on the adipose:plasma partition coefficients of 24 PCBs were used in a regression analysis to find an expression for the adipose:plasma partition coefficient as a function of molecular structure. Using stepwise regression, it was found that three simple structural descriptors were sufficient to predict adipose:plasma partition coefficients for all 209 PCB congeners. Data on the distribution of PCBs among blood components were used to derive the adipose:blood partition coefficient from the adipose: plasma partition coefficient. The lipid contents of liver, muscle, and skin were used to derive the tissue: blood partition coefficient for those tissues from the adipose:blood partition coefficient. These results allow for the calculation of tissue:blood partition coefficients for liver, skin, muscles, and fat for all 209 PCB congeners.

Pastino GM, Asgharian B, Roberts K, Medinsky MA, Bond JA. **A comparison of physiologically based pharmacokinetic model predictions and experimental data for inhaled ethanol in male and female B6C3F1 mice, F344 rats, and humans.** Toxicol Appl Pharmacol 1997;145(1):147-57.

Ethanol is added to unleaded gasoline as an oxygenate to decrease carbon monoxide automobile emissions. This introduces inhalation as a new possible route of environmental exposure to humans. Knowledge of the pharmacokinetics of inhaled ethanol is critical for adequately assessing the dosimetry of this chemical in humans. The purpose of this study was to characterize the pharmacokinetics of inhaled ethanol in male and female B6C3F1 mice and F344 rats and to develop a physiologically based pharmacokinetic (PBPK) model for inhaled ethanol in mice, rats, and humans. During exposure to 600 ppm for 6 hr, steady-state blood ethanol concentrations (BEC) were reached within 30 min in rats and within 5 min in mice. Maximum BEC ranged from 71 microM in rats to 105 microM in mice. Exposure to 200 ppm ethanol for 30 min resulted in peak BEC of approximately 25 microM in mice and approximately 15 microM in rats. Peak BEC of about 10 microM were measured following exposure to 50 ppm in female rats and male and female mice, while blood ethanol was undetectable in male rats. No sex-dependent differences in peak BEC at any exposure level were observed. Species-dependent differences were found following exposure to 200 and 600 ppm. A blood flow limited PBPK model for ethanol inhalation was developed in mice, rats, and humans which accounted for a fractional absorption of ethanol. Compartments for the model included the pulmonary blood and air, brain, liver, fat, and rapidly perfused and slowly perfused tissues. The PBPK model accurately simulated BEC in rats and mice at all exposure levels, as well as BEC reported in human males in previously published studies. Simulated peak BEC in human males following exposure to 50 and 600 ppm ranged from 7 to 23 microM and 86 and 293 microM, respectively. These results illustrate that inhalation of ethanol at or above the concentrations expected to occur upon refueling results in minimal BEC and are unlikely to result in toxicity.

Pentikis HS, Henderson JD, Tran NL, Ludden TM. **Bioequivalence: individual and population compartmental modeling compared to the noncompartmental approach**. Pharm Res 1996 Jul;13:1116-21.
IPA COPYRIGHT: ASHP Individual compartmental and population compartmental methods for determining bioequivalence were compared with the traditional noncompartmental approach using data from 3 bioequivalence studies of chlorthalidone. Individual compartmental modeling and population compartmental modeling techniques performed well on this routine set of bioequivalence data that displayed simple pharmacokinetic properties. A direct assessment of the analytical methods was made by comparing the final estimates and 90% confidence intervals for the test to reference ratios (T/R) of area under the curve (AUC) and Cmax. The final estimates and 90% confidence intervals for AUC T/R and Cmax T/R were similar and suggested consistency of results, independent of the method used.

Pereira CM, Tam YK, Coe JY, Olley PM, Collins-Nakai RL. **Pharmacokinetic-pharmacodynamic modelling for captopril in healthy anesthetized piglets**. Biopharm Drug Dispos 1996 Jul;17:365-72.
IPA COPYRIGHT: ASHP To investigate the relationship between the pharmacokinetics and pharmacodynamics of captopril, piglets received 0.2 mg/kg of intravenous captopril; blood samples were taken frequently and analyzed for pharmacokinetic parameters and hemodynamics were monitored. The pharmacokinetic parameters and hemodynamic response were similar to those in humans. A parametric 2-compartment linear pharmacokinetic-pharmacodynamic model was established that describes plasma captopril concentration and aortic pressure relationship.

Piotrovskij V, Van Peer A. **A model with separate hepato-portal compartment (first-pass model): fitting to plasma concentration-time profiles in humans**. Pharm Res 1997;14(2):230-7.

Piscitelli SC, Reiss WG, Figg WD, Petros WP. **Pharmacokinetic studies with recombinant cytokines. Scientific issues and practical considerations.** Clin Pharmacokinet 1997;32(5):368-81.
Advances in molecular biology and recombinant DNA technology have led to the development of cytokines as therapeutic agents for a variety of disease states. The pharmacokinetic analysis of cytokines involves the understanding of analytical methods capable of detecting these agents in biological fluids and recognition of several factors which may have an impact on the cytokine concentration-time curves. Enzyme-linked immunosorbent assays (ELISA) have become the most common method of detection and commercial kits are available for a wide variety of cytokines. Monoclonal antibody products are sensitive, have minimal cross-reactivity and are relatively inexpensive when compared with high performance liquid chromatography (HPLC). However, the primary limitation of these assays is their inability to measure biologically active protein. Conversely, bioassays do measure a biological event (i. e. proliferation or cytotoxicity) but are generally not used for cytokine analysis because of their high cost, long assay completion time, lack of specificity, poor sensitivity and influence of environmental conditions on the outcome. The pharmacokinetic profile of recombinant cytokines is influenced by a number of variables: endogenous production, circulating soluble receptors and cell-associated receptors, immunocompetence and antibody production against the cytokine all may influence the disposition of the agent. Thus, pharmacokinetic modelling of cytokines may involve complex models capable of characterising these nonlinear processes and resulting effects. The route of administration is an important variable since cytokines administered by subcutaneous injection may be partially metabolised by proteases present in the subcutaneous tissue. Other methods to simplify cytokine delivery are being actively investigated and include formulations for inhalation, topical and oral administration. A variety of cytokines (including interferon-alpha, interleukin-6 and tumour necrosis factor) are capable of inhibiting cytochrome P450 hepatic enzymes and, therefore, possess the potential to cause drug-cytokine interactions. Inhibition has been demonstrated in several in vitro systems and animal models, although clinical data are currently limited. An increased understanding of the many factors which can alter the analysis and pharmacokinetics of cytokines is essential to the design of optimal dosage regimens.

Portier C, Kohn M. **A biologically based model for the carcinogenic effects of 2,3,7,8-TCDD in female Sprague-Dawley rats**. Organohalogen Compd 1996;29:222-7.
CBAC COPYRIGHT: CHEM ABS A review with 11 refs. is given on mechanistic models involving hepatic focal lesions and carcinogenesis and the adequacy of the 2-stage model for risk assessment of exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in rats.

Raghunathan K, Schmitz JC, Priest DG. **Disposition of leucovorin and its metabolites in dietary folic acid-deplete mice - comparison between tumor, liver, and plasma.** Cancer Chemother Pharmacol 1997;40(2):126-30.
PURPOSE: A comprehensive pharmacokinetic study of leucovorin (5-formyltetrahydrofolate, 5-HCO-FH4) and its metabolites was conducted in plasma, liver and implanted tumor tissue from mice maintained on a low folic acid diet. While it has been previously demonstrated that the antitumor activity of fluorouracil (FU) can be potentiated by 5-HCO-FH4, the optimum time for administration of

FU after 5-HCO-FH4, to maximally elevate the active folate metabolite methylenetetrahydrofolate in tumor has not been established. Human plasma studies have defined the pharmacokinetics of circulating 5-HCO-FH4 and its metabolites.

Roy A, Weisel CP, Gallo MA, Georgopoulos PG. **Studies of multiroute exposure-dose reconstruction using physiologically based pharmacokinetic models**. Toxicol Ind Health 1996;12(2):153-63.
BIOSIS COPYRIGHT: BIOL ABS. RRM RESEARCH ARTICLE HUMAN MODELS AND SIMULATIONS MATHEMATICAL MODEL CHLOROFORM POLLUTANT PHARMACOKINETICS PHARMACODYNAMICS EXHALED BREATH CONCENTRATION DERMAL ABSORPTION POLLUTION TOXICOLOGY NON-LINEAR CAUSE-EFFECT RELATIONSHIP EXPOSURE-DOSE RECONSTRUCTION.

Rybak MJ, Houlihan HH, Mercier R, Kaatz GW. **Pharmacodynamics of RP 59500 (Quinupristin-dalfopristin) administered by intermittent versus continuous infusion against Staphylococcus aureus-infected fibrin-platelet clots in an in vitro infection model**. Antimicrob Agents Chemother 1997 Jun;41(6):1359-63.

Rydberg T, Joensson A, Karlsson MO, Melander A. **Concentration-effect relations of glibenclamide and its active metabolites in man: modeling of pharmacokinetics and pharmacodynamics**. Br J Clin Pharmacol 1997;43(4):373-81.

Sands H, Li J, Duggaraju R, Kolan HR, Donegan M, Elove GA, Thakur ML. **Biodistribution and pharmacokinetics of 131I-labeled LEX 032, a recombinant hybrid of antichymotrypsin**. Drug Metab Dispos 1997;25(5):631-6.
CBAC COPYRIGHT: CHEM ABS Pharmacokinetic and biodistribution studies were conducted in rats on a novel serine protease inhibitor, LEX 032, that was radiolabeled with 131I by the Bolton-Hunter reagent. LEX 032, a genetically engineered recombinant human nonglycosylated serpin, has been shown to have antiinflammatory properties in a no. of animal models of inflammation and reperfusion injury. When 131I-LEX 032 was injected i.v., a rapid whole body clearance of radioactivity was seen. Blood clearance followed a similar pattern. Forty-eight hours postinjection, 200 .+-. 0.65% of the administered dose remained in the body. Greater than 59% of the radioactivity was excreted in the urine within the first 24 h. Little radioactivity was found in the feces. With the exception of the thyroid, no significant organ-related uptake was noted. Radioactivity in the liver peaked at 20 min postinjection, with 1.00 .+-. 0.13% administered dose/g and .apprx.10% administered dose in the whole liver. At 1 h, uptake in the kidney (9.30 .+-. 1.52% administered dose/g) was the higher among all tissues, except for the thyroid. Gamma camera images were consistent with the biodistribution pattern. Pharmacokinetics and biodistribution were not affected by the dose of LEX 032 and were quite different from those of glycosylated wild type antichymotrypsin. These data indicate that LEX 032 exhibits the pharmacokinetics expected of a nonglycosylated 45 kDa protein.

Sato A, Perlas E, Ben-Menahem D, Kudo M, Pixley MR, Furuhashi M, Hsueh AJ, Boime I. **Cystine knot of the gonadotropin alpha subunit is critical for intracellular behavior but not for in vitro**

biological activity. J Biol Chem 1997;272(29):18098-103.
The common alpha subunit of glycoprotein hormones contains five disulfide bonds. Based on the published crystal structure, the assignments are 7-31, 59-87, 10-60, 28-82, and 32-84; the last three comprise the cystine knot, a structure also seen in a variety of growth factors. Previously, we demonstrated that the efficiency of secretion and the ability to form heterodimers by alpha subunits bearing single cysteine residue mutants in the cystine knot were significantly reduced. These results suggested that the cystine knot is critical for the intracellular integrity of the subunit. To assess if the presence of the free thiol affected the secretion kinetics, we constructed paired cysteine mutants of each disulfide bond of the alpha subunit. The secretion rate for these monomers was comparable with wild type except for the alpha-10-60 mutant, which was 40% lower. The recovery of the alpha7-31 and alpha59-87 mutants was greater than 95%, whereas for the cystine knot mutants, it was 20-40%. Co-expression of the wild-type chorionic gonadotropin beta subunit with double cysteine mutants did not enhance the recovery of alpha mutants in the media. Moreover, compared with wild-type, the efficiency of heterodimer formation of the alpha10-60 or alpha32-84 mutants was less than 5%. Because subunit assembly is required for biological activity, studies on the role of these disulfide bonds in signal transduction were not possible. To bypass the assembly step, we exploited the single chain model, where the alpha and beta subunits are genetically fused. The recovery of secreted tethered gonadotropins bearing mutations in the cystine knot was increased significantly. Although dimer-specific monoclonal antibodies discriminated the conformation of single chain alpha10-60 and alpha32-84 mutants from the native heterodimer, these mutants were nevertheless biologically active. Thus, individual bonds of cystine knot are important for secretion and heterodimer formation but not for in vitro bioactivity. Moreover, the data suggest that the native heterodimer configuration is not a prerequisite for receptor binding or signal transduction.

Sauer JM, Smith RL, Bao J, Kattnig MJ, Kuester RK, McClure TD, Mayersohn M, Sipes IG. **Oral and topical absorption, disposition kinetics, and the metabolic fate of trans-methyl styryl ketone in the male Fischer 344 rat.** Drug Metab Dispos 1997;25(6):732-9.
trans-Methyl styryl ketone (MSK; trans-4-phenyl-3-buten-2-one) is a beta-unsaturated ketone that has a wide range of uses in industry and is present in numerous consumer products. Although MSK has been shown to be positive in several in vitro mutagenic assays, it does not seem to be overtly toxic in animal models. This lack of toxicity may relate to its poor absorption and/or rapid elimination. However, little is known about the fate of MSK in the body. Studies were conducted to characterize the absorption, and disposition kinetics of MSK after intravenous, oral, and topical administration to male Fischer 344 rats. After intravenous administration of [14C]MSK (20 mg/kg, 120 microCi/kg), blood concentration-time data could be characterized with a biexponential equation and apparent first-order elimination kinetics. The following pharmacokinetic parameter values were obtained (mean +/- SD): terminal disposition half-life, 17.7 +/- 0.08 min; apparent steady-state volume of distribution, 0.89 +/- 0.14 liters/kg; systemic body clearance, 68.9 +/- 10.0 ml/kg *min; and mean residence time, 13.1 +/- 2.2 min. Within 48 hr, 95.5% of the dose was excreted in the urine and 2.7% in the feces. The major blood metabolite after intravenous administration was identified by GC/MS as the 4-phenyl-3-buten-2-ol (methyl styryl carbinol). After oral administration of [14C]MSK (200 mg/kg, 100 microCI/kg), approximately 96.6% of the dosed radioactivity was recovered in the urine and 4.8% in the faces within 48 hr. Major urinary metabolites identified by LC-MS/MS and quantified by HPLC radioassay were N-phenylacetyl-L-

glycine (64.9% of dose) and N-benzyl-L-glycine (9.9% of dose). Parent compound could not be detected in the blood after oral administration, and 14C-equivalents in the blood never exceeded 1.3% of the dose. Results suggest near-total presystemic elimination of the oral dose. After topical application of [14C]MSK (250 mg/kg, 50 microCi/kg), > 60% of the dose was absorbed, and the majority of the dose was excreted into the urine (55% of dose) in the form of metabolites. Urinary metabolites were similar to those described after oral administration. 14C-equivalents were not detected in the blood at any time after topical administration. These results indicate that MSK is almost totally metabolized before systemic distribution after oral or topical administration. The systemic exposure dose of MSK seems to be exceedingly low at the doses studied herein.

Shao R, Tang R, Wang Z, Wu W, Wang F. **[Pharmacokinetics of magnesium glutamate in rabbits]**. Zhongguo Yiyao Gongye Zazhi 1996;27(9):395-6. (Chi)
CBAC COPYRIGHT: CHEM ABS After i.v. injection of magnesium glutamate soln., the serum magnesium level of rabbits was detd. by colorimetrically methods using magnesium reagent at the wavelength of 540 nm. Program package was utilized for estn. of pharmacokinetic parameters. 2 Compartment model was adopted in computer simulation. The pharmacokinetic parameters were as follows: alpha = 4.49 h-1, beta = 0.07 h-1, T1/2alpha = 0.15 h, T1/2beta = 9.48 h, R12 = 2.16 h-1, R21 = 2.23 h-1, and R10 = 0.15 h-1. The parameters were compared with those of magnesium sulfate.

Tannheimer SL, Barton SL, Ethier SP, Burchiel SW. **Carcinogenic polycyclic aromatic hydrocarbons increase intracellular Ca2+ and cell proliferation in primary human mammary epithelial cells.** Carcinogenesis 1997;18(6):1177-82.

Traxler P, Furet P, Mett H, Buchdunger E, Meyer T, Lydon N. **Design and synthesis of novel tyrosine kinase inhibitors using a pharmacophore model of the ATP-binding site of the EGF-R**. J Pharm Belg 1997;52(2):88-96.
CBAC COPYRIGHT: CHEM ABS A review with 29 refs. One of the most promising targets for the rational design of anti-cancer drugs is the family of the EGF-receptor protein tyrosine kinases.

Wang X, Santostefano MJ, Evans MV, Richardson VM, Diliberto JJ, Birnbaum LS. **Receptor-incorporated physiologically based pharmacokinetic model for TCDD distribution in rat**. Organohalogen Compd 1996;29:389-93.

Washburn BS, Rein KS, Baden DG, Walsh PJ, Hinton DE, Tullis K, Denison MS. **Brevetoxin-6 (PbTx-6), a nonaromatic marine neurotoxin, is a ligand of the aryl hydrocarbon receptor.** Arch Biochem Biophys 1997;343(2):149-56.
Brevetoxins (PbTx) are a family of marine polyether toxins that exert their toxic action by activating voltage-sensitive sodium channels. Two forms of brevetoxin, PbTx-2 and -3, induce hepatic cytochrome P4501A1, measured as ethoxyresorufin O-deethylase (EROD) activity, in redfish and striped bass. P4501A1 induction is transcriptionally regulated through the binding of a ligand, typically a planar aromatic compound, to the aryl hydrocarbon receptor (AhR) and subsequent complex formation with the dioxin response element (DRE), an upstream regulatory region of the CYP1A1 gene. To determine if

PbTx, a nonaromatic compound, induced EROD by this mechanism, two sets of experiments were performed. Initially, saturation binding assays with PbTx-2, -3, and -6 were carried out to determine if PbTx-2, -3, or -6 was an AhR ligand. Results showed that PbTx-6 inhibited specific binding of dioxin to the AhR, whereas PbTx-2 and -3 had no effect. Subsequently, gel retardation assays showed that PbTx-6 caused a concentration-dependent increase in AhR-DRE complex formation. The most abundant and neurotoxic forms of brevetoxin, PbTx-2 and -3, did not appear to be involved in this process. However, PbTx-6, the epoxide which is a likely biotransformation product, is at least one of the forms of PbTx involved in EROD induction.

Wu ZY, Smithers BM, Roberts MS. **Melphalan dosing regimens for management of recurrent melanoma by isolated limb perfusion: application of a physiological pharmacokinetic model based on melphalan distribution in the isolated perfused rat hindlimb.** Melanoma Res 1997; 7(3):252-64. The optimal dosing schedule for melphalan therapy of recurrent malignant melanoma in isolated limb perfusions has been examined using a physiological pharmacokinetic model with data from isolated rat hindlimb perfusions (IRHP). The study included a comparison of melphalan distribution in IRHP under hyperthermia and normothermia conditions. Rat hindlimbs were perfused with Krebs-Hen-seleit buffer containing 4.7% bovine serum albumin at 37 or 41.5 degrees C at a flow rate of 4 ml/min. Concentrations of melphalan in perfusate and tissues were determined by high performance liquid chromatography with fluorescence detection. The concentration of melphalan in perfusate and tissues was linearly related to the input concentration. The rate and amount of melphalan uptake into the different tissues was higher at 41.5 degrees C than at 37 degrees C. A physiological pharmacokinetic model was validated from the tissue and perfusate time course of melphalan after melphalan perfusion. Application of the model involved the amount of melphalan exposure in the muscle, skin and fat in a recirculation system was related to the method of melphalan administration: single bolus > divided bolus > infusion. The peak concentration of melphalan in the perfusate was also related to the method of administration in the same order. Infusing the total dose of melphalan over 20 min during a 60 min perfusion optimized the exposure of tissues to melphalan.

Wyatt S, Pinon LG, Ernfors P, Davies AM. **Sympathetic neuron survival and TrkA expression in NT3-deficient mouse embryos**. Embo J 1997;16(11):3115-23.

Yang CW, Borowitz JL, Gunasekar PG, Isom GE. **Cyanide-stimulated inositol 1,4,5-trisphosphate formation: an intracellular neurotoxic signaling cascade.** J Biochem Toxicol 1996;11(5):251-6. Cyanide-induced neurotoxicity is associated with altered cellular Ca2+ homeostasis resulting in sustained elevation of cytosolic Ca2+. In order to characterize the effect of cyanide on intracellular signaling mechanisms, the interaction of KCN with the inositol 1,4,5-trisphosphate Ca2+ signaling system was determined in the PC12 cell line. KCN in the concentration range of 1.0-100 microM produced a rapid rise in intracellular IP3 levels (peak level occurred within 60 sec); 10 microM KCN elevated intracellular levels of IP3 to 148% of control levels. This response was mediated by phospholipase C (PLC) since U73122, a specific PLC inhibitor, blocked the response. Removal of Ca2+ from the incubation medium and chelation of intracellular Ca2+ with BAPTA partially attenuate the cyanide-stimulated IP3 generation, showing that the response is partially Ca2+ dependent. Also, treatment of cells with nifedipine or LaCl3, Ca2+ channel blockers, partially blocked the generation of

IP3. This study shows that cyanide in concentrations as low as 1 microM stimulates IP3 generation that may be mediated by receptor and nonreceptor IP3 production since they have differential dependence on Ca2+. It is proposed that this response is an early intracellular signaling action that can contribute to altered Ca2+ homeostasis characteristic of cyanide neurotoxicity.

Yang J, Wang M, Lu X, Ke F. **[Effect of birchdust on viability of alveolar macrophages and superoxide produced by alveolar macrophages in vitro]**. Hua Hsi I Ko Ta Hsueh Hsueh Pao 1996;27 (1):97-9. (Chi)
Rabbit alveolar macrophages (AM) in vitro were used as a model in this wooddust toxicological experiment to study the effect of birchdust with different concentrations and exposure durations on the viability of AM and superoxide produced by AM. The results showed that the viability rates in three birchdust groups (400, 800, 1600 micrograms/ml) gradually decreased when the concentration at all the designed exposure durations (12, 18, 24 h) increased except the rates for the 6-hour period. On the contrary, the contents of superoxide (O2-.) at the first two durations (6, 12 h) increased rapidly and reached their highest level at the 18 hours. Moreover, the rapid increase of O2-. preceded the declination of viability rates, suggesting that birchdust toxicity in AM in vitro be probably related to dust-induced O2-. produced by AM.

Zeilmaker MJ, Van Eijkeren JC. **Mathematical modeling of Ah-receptor regulated P 450 gene expression and its application in the risk assessment of 2,3,7,8-TCDD**. Organohalogen Compd 1996;30:286-9.

Zhang Z, Tsuneji N. **A study on the multi-compartment linear circulation pharmacokinetic model for the targeting drug delivery system**. J Chin Pharm Sci 1996;5(2):81-7.
CBAC COPYRIGHT: CHEM ABS A multi-compartment linear circulation pharmacokinetic model (MCLCPM) for the targeting drug delivery system was established and the function formula of the drug concn.-time curve in blood and the target organ were derived. The drug concn.-time curve of the target organ and the drug pharmacokinetic parameters was plotted out with ref. to the data of drug concn. in blood. The validity of this model was checked by the liver targeting nanoparticles in animal expts. Based on the liver drug concn.-time curves calcd. by the function formula of the drug in the target organ, the pharmacokinetic behavior and parameters of the drug in the target organ (liver) were analyzed and obtained by statistical moment. The results suggest that the relative targeting index is valid in quant. evaluation of the targeting drug delivery systems.

Zhu H, Baxter LT, Jain RK. **Potential and limitations of radioimmunodetection and radioimmunotherapy with monoclonal antibodies.** J Nucl Med 1997;38(5):731-41.
Recently, we developed a physiologically based pharmacokinetic model capable of predicting antibody biodistribution in humans by scaling up from mice. By applying this model to anticarcinoembryonic antigen murine antibody ZCE025, we address several critical issues in radioimmunodetection and radioimmunotherapy, including the optimal antibody doses, the desirable antibody form for cancer detection, the optimal combinations of antibody forms and radionuclides for cancer treatment and the effectiveness of the modality. METHODS: Under the baseline conditions of a standard 70-kg man with a 20-g tumor embedded in the liver, the model was used to: (a) estimate absorbed doses in tumor and

normal tissues, (b) determine dose-dependent antibody uptake in the tumor, (c) simulate tumor-to-background antibody concentration ratio and (d) calculate therapeutic ratios for different antibody forms and radionuclides. Sensitivity analysis further enabled us to determine antibody delivery barriers and to assess the modality under average and favorable tumor physiological conditions. RESULTS: By using ZCE025 under the baseline conditions, the model found that Fab was the most suitable form for cancer diagnosis, while 131l combined with F(ab')2 provided the highest tumor-to-bone marrow therapeutic ratio for cancer treatment. Sensitivity analysis showed that antibody permeability was the major barrier for antibody accretion in tumors. It also demonstrated that normal tissue antigen expression at a level lower than in the tumor had little effect on the therapeutic ratio. CONCLUSION: The model demonstrates that: (a) for radioimmunodetection, the most effective antibody form (Fab for ZCE025) was the lower mol weight form, yet not sensitive enough for hepatic metastasis detection; and (b) for radioimmunotherapy, a relatively fast-clearing antibody form (F(ab')2 for ZCE025) in combination with long half-life beta(-)-emitters was optimal, yet inadequate as the sole therapeutic modality for solid tumors.

PULMONARY TOXICITY

Bhattacharyya SN, Manna B, Ashbaugh P, Coutinho R, Kaufman B. **Differentiation of respiratory epithelium: the effects of retinoic acid and carcinogens on the expression of mucociliary vs. squamous phenotype.** Inflammation 1997;21(2):133-43.
BIOSIS COPYRIGHT: BIOL ABS. Changes in ultrastructural characteristics and mucin gene expression were examined in rat tracheal explants cultured in a synthetic medium : retinoic acid (RA), benzo(a)pyrene (B(a)P) and N-methyl-N-nitrosourea (NMNU). In the RA(+) cultures, no changes in either ultrastructural features or mucin gene expression were detected after 48 h incubation. After 96 h incubation, however, the ultrastructural features associated with the squamous phenotype were characteristics of cultures containing the two carcinogens and the mucin gene expression was slightly reduced. Thus, in the presence d retinoic acid, the carcinogen induced changes in cytology to the squamous phenotype were not matched by a marked loss of mucin gene expression. Explants cultured for 48h without RA and : carcinogens showed none of the cytological changes associated with onset of the squamous phenotype. While mucin mRNA was still detected, it was clearly reduced compared to 48h cultures in RA(+) medium. However, 48 h later, all explants exhibited pronounced squamous metaplasia and the mucin message decreased to trace levels. Thus, the results of these experiments with B(a)P and NMNU in RA(+) and RA(-) media indicates that at least the early carcinogen induced changes may be distinct from those associated with the retinoid pathway controlling expression of the mucin component of the mucociliary epithelium.

Dillon CT, Lay PA, Cholewa M, Legge GJ, Bonin AM, Collins TJ, Kostka KL, Shea-McCarthy G. **Microprobe X-ray absorption spectroscopic determination of the oxidation state of intracellular chromium following exposure of V79 Chinese hamster lung cells to genotoxic chromium complexes.** Chem Res Toxicol 1997;10(5):533-5.
The oxidation state of intracellular chromium has been determined directly in mammalian lung cells exposed to mutagenic and carcinogenic chromium compounds. Microprobe X-ray absorption spectroscopy (XAS) experiments on single V79 Chinese hamster lung cells showed that Cr(VI) and Cr

(V) complexes were reduced completely (>90%) to Cr(III) within 4 h of exposure of the cells. This result provides direct evidence for the hypothesis that these genotoxic oxidants react rapidly with intracellular reductants.

Stewart AG, Tomlinson PR, Wilson JW. **beta2-Adrenoceptor agonist-mediated inhibition of human airway smooth muscle cell proliferation: importance of the duration of beta2-adrenoceptor stimulation**. Br J Pharmacol 1997;121(3):361-8.

QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIPS

Anderson PL, Haglund P, Tysklind M. **Ultraviolet absorption spectra of all 209 polychlorinated biphenyls evaluated by principal component analysis.** Fresenius' J Anal Chem 1997;357(8):1088-92.
BIOSIS COPYRIGHT: BIOL ABS. The ultraviolet absorption spectra of all 209 polychlorinated biphenyls (PCBs) were recorded in the range 200-300 nm and displayed two important absorption maxima, viz., the main-band, lambdamax 200-225 nm, and the kappa-band, lambdamax 245-265 nm. By utilizing principal component analysis, substitution related spectral characteristics of the PCBs, underlying the main patterns of the spectra, were examined. Captured in the multivariate evaluation were e.g., the importance of chlorine atoms in or positions, determining the intensity and existence of the K-band, chlorine substitution in para-para position, and the total number of chlorine atoms. The measured UV-spectra of all 209 polychlorinated biphenyls provide important physico-chemical descriptors for use in future quantitative structure-activity and structure-property relationship (QSAR/ QSPR) studies.

Ashman WP, Groh MJ. **Structure-toxicity relationships of methylphosphonofluoridate analogs**. Proc Erdec Sci Conf Chem Biol Def Res 1996;931-9.

Basak SC, Grunwald GD, Niemi GJ. **Use of graph-theoretic and geometrical molecular descriptors in structure-activity relationships**. From Chem Topol Three-Dimens Geom 1997;73-116.

Benfenati E, Gini G. **Computational predictive programs (expert systems) in toxicology.** Toxicology 1997;119(3):213-25.
The increasing number of pollutants in the environment raises the problem of the toxicological risk evaluation of these chemicals. Several so called expert systems (ES) have been claimed to be able to predict toxicity of certain chemical structures. Different approaches are currently used for these ES, based on explicit rules derived from the knowledge of human experts that compiled lists of toxic moieties for instance in the case of programs called HazardExpert and DEREK or relying on statistical approaches, as in the CASE and TOPKAT programs. Here we describe and compare these and other intelligent computer programs because of their utility in obtaining at least a first rough indication of the potential toxic activity of chemicals.

Benigni R, Andreoli C, Zito R. **Prediction of rodent carcinogenicity of further 30 chemicals bioassayed by the U.S. National Toxicology Program**. Environ Health Perspect 1996;104(Suppl

5):1041-4.
Predictions based on quantitative structural activity relationships (QSAR) of the carcinogenicity of 30 chemicals undergoing rodent bioassays by the National Toxicology Program.

Brasquet C, Subrenat E, Le Cloirec P. **Selective adsorption on fibrous activated carbon of organics from aqueous solution: correlation between adsorption and molecular structure.** Water Sci Technol 1997;35(7):251-9.
BIOSIS COPYRIGHT: BIOL ABS. In industrial processes, granular activated carbon (GAC) is generally used to remove pollutants from wastewater. Recently, a new adsorbent has been explored, fibrous activated carbon (FAC). Experiments were carried out with two FACs having different specific surface areas (1500 and 1300 m2 g-1) and pore-size distributions to study adsorption of various organic compounds from aqueous solution. Results were compared with adsorption onto one GAC with a specific surface area of about 1000 m2 g-1. Classic models were applied and kinetic constants were computed. In most cases, FAC with the higher specific surface area (named CS 1501) showed better adsorption capacities and kinetics than the two other FACs. For example, adsorption velocity of benzaldehyde was 7.2 zeta 10-5 l mg-1 min-1 with CS 1501 and about 3 zeta 10-5 l mg-1 min-1 with other FACs. Furthermore, adsorption onto CS 1501 of a great number of organic compounds (aliphatic and aromatic) depended on solute molecular characteristics. For instance, solute molecular size seemed to play an important role: adsorption capacity of high molecular weight compounds (humic substances) was about 3 mg g-1, a value much lower than those of low molecular weight compounds, which were respectively 200 mg g-1 and 400 mg g-1 for phenol and benzoic acid. From experimental results, a correlation of QSAR (Quantitative Structure-Activity Relationship) type has been set up. This relationship predicts the adsorbability of organic compounds onto fibrous activated carbon from the molecular properties of these compounds.

Chen J, Wang L. **Using MTLSER model and AM1 Hamiltonian in quantitative structure-activity relationship studies of alkyl (1-phenylsulfonyl)cycloalkane-carboxylates**. Chemosphere 1997;35 (3):623-31.

Chen JW, Zhao YJ, Feng L, Han SK, Wang LS, Zhang Z. **Using AM1 Hamiltonian in quantitative structure-activity relationship studies of phenylthio-carboxylates**. Toxicol Environ Chem 1997;60(1-4):211-21.
CBAC COPYRIGHT: CHEM ABS AM1 Hamiltonian contained in the MOPAC(6.0) program package was used to compute descriptors for 18 phenylthio-carboxylates. The Modified TLSER (MTLSER) model was used to develop a QSAR equation for toxicity of these compds. The obtained equation can be used to predict toxicity to Photobacterium phosphoreum of this series of compds. The polarizability (alpha) increases the toxicity. The dipole moment (mu) and the most pos. net at. charge on a hydrogen atom (qH+) decrease the toxicity. The advantages were shown of the MTLSER descriptors: they permit near a priori prediction of toxicity; they can be easily and precisely obtained by computation instead of expt., thus a large amt. of expenses and time can be saved; and they have clear physicochem. interpretations, and interpretation of the correlation equations can suggest modes of interaction between toxicants and organisms.

Chen X, Wang L, Hu S, Lin X, Wu J. **[Improvement of constellation graph and its application in the identification of quantitative structure-activity relationship for fluorinated organic pesticides]**. Jisuanji Yu Yingyong Huaxue 1997;14(1):38-43. (Chi)

Chilmonczyk Z, Bogdal M, Mazgajska M, Cybulski J, Lewandowska U. **Structure-activity relationship in a series of new 1-(2-pyrimidinyl)piperazine derivatives with hypnotic activity.** Pol J Pharmacol 1996;48(4):431-40.
Preparation, biological properties and QSAR of new derivatives of 1-[4-(2-pyrimidinyl)-1-piperazinyl]-1, 3-butandione (11-13, and 15-18) and 3-[4-(2-pirimidinyl)-1-piperazinyl]-3-oxopropanoate (20-22) exhibiting hypnotic activity in mice are reported. The best therapeutic indices (TI = LD50/ED50) 4.7 and 9.4 for po and ip administration, respectively, were found for 1-[4-(2-pyrimidinyl)-1-piperazinyl]-2-n-pentyl-1,3-butandione (15). QSAR studies showed that the biological activity grows initially with an increase in lipophilicity to drop dramatically for log P > 2.5.

Chung K, Kirkovsky L, Kirkovsky A, Purcell WP. **Review of mutagenicity of monocyclic aromatic amines: quantitative structure-activity relationships**. Mutat Res 1997;378(1):1-16.
CBAC COPYRIGHT: CHEM ABS A review with 75 refs. Monocyclic arom. amines (MAAs) are environmental pollutants. Many of them are genotoxic and impose hazards to human health. The mutagenicity of more than 80 of these amines was reviewed with primary emphasis on evaluation by the Ames Salmonella/microsome testing system. Many amines are mutagenic in Salmonella tester stains TA98 and TA100, but S9 mix is required for activity for most of the active ones. 2,4-Diaminotoluene, 2,4-diaminoethylbenzene, and a few amines contg. a nitro-group are direct mutagens. There are several quant. structure-activity relation (QSAR) models which rationalize mutagenicity of many arom. amines and several parameters, such as the LUMO energy (ELUMO), HOMO energy (EHOMO), and hydrophobicity that are important. What factors det. the min. requirement for the compd. to be mutagenic and what factors det. the extent of mutagenicity suggest questions for further study.

Costa MC, Gaudio AC, Takahata Y. **A comparative study of principal component and linear multiple regression analysis in SAR and QSAR applied to 1,4-dihydropyridine calcium channel antagonists (nifedipine analogs)**. Theochem 1997;394(2-3):291-300.
CBAC COPYRIGHT: CHEM ABS The method of principal component anal. was shown to be capable of classifying 1,4-dihydropyridine derivs. into high active and low active groups for various different sets of compds. Mainly quantum chem. parameters were used with this method. The kind of parameters employed by the method were different to those employed by linear multiple.

Dai S, Song W, Li T, Zhuang Y. **S[tudy on the structure-biodegradability relationships of azo dyes]**. Huanjing Kexue Jinzhan 1996;4(6):1-9. (Chi)
CBAC COPYRIGHT: CHEM ABS Review structure biodegradability azo dye wastewater;Azo dyes The structure-biodegradability relationships of azo dyes and biol. wastewater treatment;Biodegradable materials.

Dove S, Buschauer A. **Stepwise leave-one-isomer-out free-Wilson approaches as preprocessing**

tools in QSAR analysis of racemates. Quant Struct-Act Relat 1997;16(1):11-9.
CBAC COPYRIGHT: CHEM ABS QSAR anal. of racemates is complicated if specific substituent-receptor interactions and, by that, specific spatial fits to the binding site result in individual but unknown activity differences of enantiomers, and even in structure-dependent changes of which is the more active configuration. In a first approxn., additivity of substituent contributions should be assumed instead of major conformational effects. Then, Free-Wilson anal. (FWA) can be used as preprocessing tool to reduce a starting set of all pairs of enantiomers into a final series of the probably (more) active configurations. A stepwise leave-one-isomer-out approach is applied, where the model is successively improved by checking all remaining pairs and leaving out one enantiomer, detd. by a special criterion of poorest prediction, in each step. The final model is given by the maximal F value. This approach was applied to histamine H1 antagonistic activity (pKB, guinea pig ileum) of 19 racemic and six non-chiral phenyl-halogenated N-(diphenylpropyl)-N'-(imidazolylpropyl)guanidines. Based on only eight variables because of additivity of meta and para.

Dvorsky R, Balaz S, Sawchuk RJ. **Kinetics of subcellular distribution of compounds in simple biosystems and its use in QSAR.** J Theor Biol 1997;185(2):213-22.
An explicit expression describing the kinetics of distribution and, in some cases, biological activity in drugs and other anthropogenic chemicals in a four-compartment system consisting of a catenary chain of alternating aqueous and lipoid phases is derived. Substitution of the transport rate parameters by their appropriate relations to the reference partition coefficient converts the kinetic equations into the quantitative structure-time-activity relationship (QSTAR) or its fixed-time equivalent, QSAR. The resulting expression describes satisfactorily the published data on antibacterial activity of n-alkyl amines as a function of their hydrophobicity. The hydrophobicity-activity profile consists of two or three smoothly connected linear parts. The model can be used in drug design, pharmacokinetics, and toxicology for description of the distribution of compounds in simple biosystems and in environmental sciences to predict the fate and effects of.

Estrada E, Rodriguez L. **Decomposition of the Wiener number into contributions coming from homodistant pairs of vertices. Definition and a QSAR application**. J Serb Chem Soc 1997;62(3):199-205.
CBAC COPYRIGHT: CHEM ABS The Wiener no. is expressed in terms of homodistant pairs of vertices of the mol. graph. Two pairs of vertices are called homodistant if they are sepd. by the same distance, i.e., if they correspond to equal entries in the distance matrix. The nos. of homodistant pairs of vertices of different order, i.e., the counts of ones, twos, threes, etc., in the distance matrix, are shown to differ from the path nos. of graphs of mols. contg. cycles. These nos. are used to describe the antiviral activity of benzimidazole derivs. The model found contains two independent variables: one related to global topol. features of mols., the other related to possible specific interactions with biol. receptors. The quant. structure-activity model found is compared with other models based on mol. connectivity indexes, the original Wiener no. and the no. of paths of different length. The present approach has some strong and weak features, relative to other QSAR studies of specific drug-receptor interactions.

Fang N, Rowlands JC, Casida JE. **Anomalous structure-activity relationships of 13-homo-13-oxarotenoids and 13-homo-13-oxadehydrorotenoids**. Chem Res Toxicol 1997;10(8):853-8.

CBAC COPYRIGHT: CHEM ABS Cube resin, used as an insecticide/miticide and piscicide, contains in decreasing amts. rotenone (I), deguelin (II), the 6a,12a-dehydro derivs. of rotenone (III) and deguelin (IV), and the newly-discovered 13-homo-13-oxa-6a,12a-dehydro analogs [referred to as oxadehydrorotenone (V) and -deguelin (VI)]. These six rotenoids were compared for potency as inhibitors of NADH:ubiquinone oxidoreductase activity and for organismal toxicity to mosquito larvae, goldfish, and mice and cytotoxicity in three mammalian cell lines (Hepa 1C1C7, MCF 7, and NB 41A3). Although rotenoids 3-6 contribute very little to the overall activity of cube resin, there were two surprising aspects to the structure-activity relationships. First, I was 7-15-fold more active than II in the cytotoxicity assays of 4-day duration, but not in the other systems. This difference in cytotoxicity is not due to specificity at the oxidoreductase target, but instead to more extensive cytochrome P 450-dependent (piperonyl butoxide-sensitive) detoxification of II than of I. Second, the obsd. potency increase on conversion of dehydrorotenone to either rotenone or oxadehydrorotenone suggests that combining both structural changes to form cis-13-homo-13-oxarotenone (VII) might result in maximal activity. Accordingly, V was reduced with diisobutylaluminum hydride to the trans-isomer (VIII) and then epimerized with aq. pyridine to the cis-isomer VII of the same configuration as I. Surprisingly, VII was much less active than I. This is rationalized on the basis of conformational changes in the B/C ring system and decreasing dihedral angle (detd. by X-ray crystallog. and/or mol. modeling) between the A and D rings that follow the potency order, i.e., rotenoids I and II > oxadehydrorotenoids V and VI > trans- and cis-oxarotenoids VIII and VII > dehydrorotenoids III and IV. Thus, the novel oxarotenoids and oxadehydrorotenoids help define the conformation optimal for NADH:ubiquinone oxidoreductase inhibition and toxicity.

Ferguson AM, Heritage T, Jonathon P, Pack SE, Phillips L, Rogan J, Snaith PJ. **EVA: a new theoretically based molecular descriptor for use in QSAR/QSPR analysis**. J Comput Aided Mol Des 1997;11(2):143-52.
CBAC COPYRIGHT: CHEM ABS A new descriptor of mol. structure, EVA, for use in the derivation of robustly predictive QSAR relationships is described. It is based on theor. derived normal coordinate frequencies, and has been used extensively and successfully in proprietary chem. discovery programs within Shell Research. As a result of informal dissemination of the methodol., it is now being used successfully in related areas such as pharmaceutical drug discovery. Much of the exptl. data used in development remain proprietary, and are not available for publication. This paper describes the method and illustrates its application to the calcn. of nonproprietary data, log Pow, in both explanatory and predictive modes. It will be followed by other publications illustrating its application to a range of data derived from biol. systems.

Font M, Monge A, Ruiz I, Heras B. **Structure-activity relationships in quinoline reissert derivatives with HIV-1 reverse transcriptase inhibitory activity**. Drug Des Discov 1997;14(4):259-72.

Fradera X, Amat L, Besalu E, Carbo-Dorca R. **Application of molecular quantum similarity to QSAR**. Quant Struct Act Relat 1997;16(1):25-32.
CBAC COPYRIGHT: CHEM ABS Mol. Quantum Similarity Measures (MQSM), which allow quant. comparison between mol. electronic d. distributions, are investigated as a potential source of QSAR parameters. By computing the MQSM for all possible mol. pairs in a given mol. set, a Similarity Matrix

is obtained, contg. relevant information about the structural relationships within the set. Approx. Overlap-like MQSM using several computational levels are employed to obtain similarity matrixes for three mol. sets, taken as test cases: 1) non-branched alkanes, 2) Baker triazines, 3) indole derivs. The similarity matrixes have been analyzed by means of PCA and PLS techniques. QSAR regression models were derived in order to fit available property or biol. activity data. A reasonably good correlation between properties or activities and the similarity measures has been found.

Fujita T. **Recent success stories leading to commercializable bioactive compounds with the aid of traditional QSAR procedures**. Quant Struct Act Relat 1997;16(2):107-12.
CBAC COPYRIGHT: CHEM ABS A review with 26 refs. New successful applications of the Hansch-type traditional QSAR to key steps in designing novel bioactive compds. are reviewed. Studies for a benzhydrylbenzylpiperazine antimigraine agent (lomerizine), azole-type agricultural fungicides (metconazole and ipconazole), and a biphenylyloxobutanoic acid antiinflammatory agent (flobufen) were taken as the examples. Structural optimizations were nicely made by using the QSAR information sometimes along with findings obtained from 3D mol. modeling studies and/or hypotheses proposed for metabolic fates. The two azole fungicides were launched in 1994. The antimigraine and antiinflammatory agents are expected to be commercialized soon.

Gheodoridis G. **Structure-activity relationships of herbicidal aryltriazolinones**. Pestic Sci 1997;50 (4):283-90.
CBAC COPYRIGHT: CHEM ABS A series of substituted aryltriazolinones, known to inhibit protoporphyrinogen oxidase, were prepd. and their structure-activity requirements at positions 4 and 5 of the arom. ring investigated. A QSAR equation obtained for substituents at the 5 position identified the hydrophobicity term pi and the Sterimol min. width B1 as the two parameters affecting in-vitro biol. activity. Greenhouse preemergence activity correlated with in-vitro activity and the hydrophobicity term pi of the substituent at the position. The phenoxy-4-oxyacetate group at arom. position 5 was an outlier and had to be considered sep. SAR anal. of substituents at arom. position 4 revealed that two different models were required to explain all obsd. substituent effects; in the first model, where the 5 position was occupied by hydrogen, the 4-chlorobenzyloxy group at arom. position 4 gave the best compd. The second model, where the 5 position of the arom. ring was occupied by a group other than hydrogen, resulted in a QSAR equation, previously derived, which links substituent effects at position 4 with pi and with the electronic para and inductive term Fp. In this model the chloro group provides optimum biol. activity. The need to sep. the aryltriazolinone herbicides into several different classes in order to explain their substituent effects at arom. positions 4 and 5 could be rationalized if more than one binding conformation, within the same binding site, is possible.

Ghoshal N, Achari B, Ghoshal TK. **A QSAR study of antiplatelet agents using artificial neural network - correlation with micelle-water partition coefficient**. Bioorg Med Chem Lett 1997;7(7):877-80.
CBAC COPYRIGHT: CHEM ABS Antiplatelet activity ex vivo data reported for 2-substituted phenyl- and benzimidazolyl-5-methyl-4-(3-pyridyl)imidazoles have been analyzed using back propagation type artificial neural network. Using micelle-water partition coeff. as an independent descriptor, a network system ($1\text{-}3_{108}1$) produced very good duplication of obsd. activities (r=0.860, SD=0.183, n=21) in the

training cycle. The results provide an improved model for prediction of antiplatelet activity.

Guilian W, Naibin B. **A study on QSAR for chlorophenols**. Fresenius Environ Bull 1996;5(1-2):67-72.
To investigate the toxicity of chlorophenol pollutants, a study was undertaken to assess the quantitative structure/activity relationship (QSAR) of chlorophenols using multivariate linear regression analysis (MLRA) and an artificial neural network (ANN) algorithm. Octanol/water partition coefficient, dissociation constant, first order valence of molecular connectivity, and molecular free surface were the molecular descriptors selected to assess chlorophenols. Toxicity values for bacteria, flounders, and daphnids were used in this study. The predictive capacity of both analytical models was assessed by comparing percent error between calculated outcomes and experimental results. ANN.

Guo M, Xu L, Hu CY, Yu SM. **Study on structure-activity relationship of organic compounds. Applications of a new highly discriminating topological index**. Match 1997;35:185-97.
CBAC COPYRIGHT: CHEM ABS A new highly discriminating topol. index EATI1 is proposed based on the adjacency matrix and used in structure-property (QSPR) and structure-activity relationship (QSAR) studies. The EATI1 values of alkanes, alcs., barbiturates, nitrogen-contg. arom. mols., and heterocyclic compds. were detd. with a regression model and correlated with a no. of physiochem. properties and biol. activities of the org. compds. The EATI1 topol. index had high structural selectivity as seen by detecting > 610,000 structures and graphs.

Huang N, Wang M, Chu F, Guo Z. **3D-QSAR studies on anticarcinogenic activity of retinoids**. J Chin Pharm Sci 1996;5(3):121-7.

Huang N, Wang M, Chu F, Guo Z. **[Studies on the structure-activity relationship of retinoids: 3D-QSAR of retinoidal anticarcinogenic activities]**. Yaoxue Xuebao 1996;31(12):932-9. (Chi)
CBAC COPYRIGHT: CHEM ABS Using comparative mol. field anal. (CoMFA), a 3D-QSAR model of anticarcinogenicity for a series of retinoids was established. The CoMFA model was validated and built by cross-validation (leave-on-out) and non-cross-validation (randomizing) techniques. The significant PLS cross-validated value RCV2 (0.905) indicated that the model could be used as a predictive tool for further design of retinoids with high activity. The activities of 3 compds. excluded from the correlation anal. were computed using this model, small residues being obtained. Based on the conformers with the lowest energy, no statistical significance existed (RCV2 = 0.420). The mol. field model as a template is able to predict activities and, to some extent, to map the topol. and physicochem. characteristics of receptor.

Jiang HL, Chen KX, Tang Y, Chen JZ, Li Y, Wang QM, Ji RY. **Theoretical and cyclic voltammetry studies on antimalarial mechanism of artemisinin (Qinghaosu) derivatives**. Indian J Chem, [B] 1997;36b(2):154-60.
CBAC COPYRIGHT: CHEM ABS Theor. calcn. methods of AM1 and PM3 have been used to calc. the electronic parameters of a series of artemisinin derivs. With Partial Least Squares (PLS) paradigm, QSAR anal. has been performed for the electronic parameters with the antimalarial activities of artemisinin derivs. The calcn. results indicate that the activity of the artemisinin derivs. is in direct

proportion to the bond strength of the peroxide bridges (.-C(10)-O(1)-O(2)-C(6)-), bond order of O(5)-C (6) and net at. charges of C(16). Accordingly, we have deduced that the pharmacophore of these compds. might be a triangle formed with the peroxide group and C(16). Moreover, the probable interaction fashion of artemisinin derivs. to the receptor has been obtained. Cyclic voltammetry detn. for some of the artemisinin derivs. has been carried out, from which cyclic voltammograms and redn. potentials have been obtained. The redn. potentials have a good correlation with the antimalarial activities, which indicate that the peroxide moiety of artemisinin analogs is important in the process of antimalarial activity. This exptl. result has been tested and verified the theor. results.

Lei X, Li C, Zhong H, Mrugacz G, Sobieszek A, Reid RE. **Quantitative structure-activity relationship studies on alkylamino 1,2-diphenylethylene compounds as calmodulin antagonists**. J Chin Pharm Sci 1996;5(4):169-73.

Liu Z, Wang L, Ni H, Kong Z. **QSAR for biotoxication of aromatic compounds**. Chin Sci Bull 1997; 42(5):380-4.
CBAC COPYRIGHT: CHEM ABS Toxic EC50, LC50, and LD50 values were used to det. QSARs.

Macina OT, Klopman G, Rosenkranz HS. **Structural basis of sensory irritation**. Inhalation Toxicol 1997;9(5):465-76.
CBAC COPYRIGHT: CHEM ABS MultiCASE (version 2.80), a structure-activity relationship expert system, was applied to a series of structural compds. evaluated for sensory irritant properties. The resulting biophores (mol. fragments responsible for the obsd. activity) were interpreted in terms of their physicochem. properties. Arom., charge-charge, and lipophilic interactions with a hypothetical receptor site are proposed. Fragment overlaps were obsd. with other databases of toxicol. interest. The MultiCASE model is useful for predictive purposes and for providing information of a mechanistic nature. Furthermore, the identified biophores can serve as starting points for in-depth exploration of the correlation between physicochem. properties and biol. activity.

Mekenyan O, Sbrana I, Turchi G. **QSAR for clastogenic effects induced by regioisomers of PAH quinones**. Polycyclic Aromat Compd 1996;11(1-4):253-60.
CBAC COPYRIGHT: CHEM ABS The exptl. results on chromosomal aberrations and spindle disturbances in mammalian liver cells for 8 regioisomers of pyrene, benzo(a)pyrene and phenanthrene quinones were compared with the AM1 calcd. stereoelectronic descriptors. The electronic structure of the parent compds. as well as the corresponding radical anions, were evaluated. Two groups of reactivity descriptors were specified evaluating the mechanisms of genotoxicity of quinones that were recently proposed by us. The first group of parameters (e.g., electronic gap) describes potency of chems. as cross-linkers of cellular macromols., whereas the second group (e.g., electronegativity, frontier orbital energies, their displacement and energy equivalence when going from quinones to the resp. intermediate anion-radicals) assesses the one electron redn. efficiency. The ordering of quinones, according to their theor. estd. reactivities, was found to be consistent with the exptl. genotoxicity data. It was concluded that genotoxic activity of studied quinones is an integrated effect of two mechanisms. The benzo(a) pyrene and pyrene quinones were predicted to be more active cross-link inducers and more effective oxidants than phenanthrene quinones.

Mor M, Bordi F, Silva C, Rivara S, Crivori P, Plazzi PV, Ballabeni V, Caretta A, Barocelli E, et al. **H3-receptor antagonists: synthesis and structure-activity relationships of para- and meta-substituted 4(5)-phenyl-2-[[2-[4(5)-imidazolyl]ethyl]thio]imidazoles.** J Med Chem 1997; 40(16):2571-8.
CBAC COPYRIGHT: CHEM ABS We report the synthesis, octanol/water partition coeff. (log P), dissocn. consts. (pKa), H3-receptor affinity (pKi in rat brain membranes, [3H]-Nalpha-methylhistamine), and H3-antagonist potency (pA2 in guinea ileum, (R)-alpha-methylhistamine) of novel H3-receptor antagonists obtained by introducing a para or meta substituent on the Ph ring of the lead compd.

Munoz FJ, Ruiz JC, Silva SL. **[Design of a QSAR information system for simulating the intensity of biological activity of some drug series]**. Rev Colomb Cienc Quim Farm 1996;25:52-9. (Spa)
CBAC COPYRIGHT: CHEM ABS A computer application SIBHA (Information System Based on Hansch's structure activity relationship model) was designed and developed. Its application (such as to drugs active on the central nervous system), allows the simulation and est. of octanol/water partition coeff. logarithm (Log P) of drugs and subsequent evaluation of biol. activity by using models reported in the literature, which render it both a potential educational alternative and a support for documenting research activities in the rational drug design process.

Noever DA, Cronise RJ, Matsos HC. **Optimized group contribution methods for predicting chemical biodegradation and eye irritancy**. Toxicol Environ Chem 1996;56(1-4):105-18.
CBAC COPYRIGHT: CHEM ABS Environmental testing increasingly has emphasized faster, more flexible methods for estg. chem. toxicity. The present work evaluates the use of computational methods to det. chem. biodegrdn. and to assess irritancy hazards. An optimized strategy begins with structure-activity relationships between functional groups in a chem. and then generalizes to assess hazards assocd. with a previously unstudied compd. formula. These compd. formulas consist of building blocks made from the functional groups, but the effects of these groups are investigated beyond a simple additive method. Both linear sums of these contributions and their corresponding non-linear fits are compared against known biodegrdn. and irritancy statistics. In this way, computation of hazards for chems. not used for modeling, but for which good literature data are available, can grade the method's performance. A correlation is found between existing in vivo databases and proposed computer prediction. These alternative computational methods share the common goal of providing accurate hazard assessments to the chem. industry without using expensive lab. animal tests.

Pajeva IK, Wiese M. **QSAR and molecular modeling of catamphiphilic drugs able to modulate multidrug resistance in tumors**. Quant Struct Act Relat 1997;16(1):1-10.
CBAC COPYRIGHT: CHEM ABS The Free-Wilson approach was applied to two groups of catamphiphilic multidrug resistance (MDR) modifiers using classical multiple linear regression (MLR) and genetic algorithms (GA) for feature selection. In the first group (17 thioxanthenes) the side chain length between the ring system and tertiary nitrogen, the type of the tertiary nitrogen substituent and the stereoisomery were found to be significant for anti-MDR activity both by MLR and GA ($r^2 = 0.803$, predictive power $Q^2 = 0.652$). In the second data set (17 phenothiazines and related drugs) the ring system type, the stereoisomery, the side chain type, and the ring substituent kind in position two

contributed significantly (r2 = 0.938 and Q2 = 0.908). The QSAR studies showed a thioxanthene ring with a -CF3 substituent in position two, a piperazine moiety with a 4-bond distance from the ring system and trans-isomery to be optimal for MDR reversal. Based on these results mol. modeling of trans-(T) and cis-flupentixol (C) was performed assuming that the 2 to 3-fold difference in MDR reversing activity of T compared to C might be related to different preferable conformations in the membrane lipid environment. Among all conformations generated by the SYBYL systematic search routine those comprising local energy min. were selected and optimized with semiempirical quantum chem. methods. The optimized conformations were compared with 1H-NMR anal. results on drug conformations in lipid environment, some of them corresponded excellently. The electrostatic and lipophilic fields of T and C were compared to identify mol. properties related to the activity difference. The results demonstrated that T and C could have a different (mirror-like) orientation entering the lipid bilayer by the ring system suggesting much better fitting of T compared to C to the lipid MDR-reversal receptor.

Ren S, Das A, Lien EJ. **QSAR analysis of membrane permeability to organic compounds**. J Drug Target 1996;4(2):103-7.
IPA COPYRIGHT: ASHP A general mathematical model involving partition coefficient, molecular weight, and hydrogen bonding was used to correlate the structures and permeation of various organic compounds through toad urinary bladder and human red blood cell membranes. Log permeability was correlated with log partition coefficient, log molecular weight, and hydrogen bonds. Log partition coefficient was the most important factor of the 3 parameters examined. It was noted that, while increased molecular weight always has a negative effect on permeability, increased hydrogen bonds can have either a slightly positive or slightly negative effect depending on the solvent and membrane systems used.

Roberts DW, Garcia MT, Ribosa I, Hreczuch W. **QSAR analysis of aquatic toxicity of ethoxylated alcohols**. Comun Jorn Com Esp Deterg 1997;27:53-63.

Rorije E, Eriksson L, Verboom H, Verhaar HJ, Hermens JL, Peijnenburg WJ. **Predicting reductive transformation rates of halogenated aliphatic compounds using different QSAR approaches**. Environ Sci Pollut Res Int 1997;4(1):47-54.
CBAC COPYRIGHT: CHEM ABS Halogenated aliph reductive dehalogenation water sediment Dehalogenation kinetics halo aliph water QSAR;Aquatic sediments Predicting reductive transformation rates of halogenated aliph. compds. in water-sediment by different QSAR approaches;Dehalogenation kinetics Predicting reductive transformation rates of halogenated aliph. compds. in water-sediment by different QSAR approaches;Dehalogenation Reductive; predicting reductive transformation rates of halogenated aliph. compds. in water-sediment by different QSAR approaches;Halo alkanes Predicting reductive transformation rates of halogenated aliph. compds. in water-sediment by different QSAR approaches;QSAR Predicting reductive transformation rates of halogenated aliph. compds. in water-sediment by different QSAR approaches Structure-activity relationship;Reduction kinetics Predicting reductive transformation rates of halogenated aliph. compds. in water-sediment by different QSAR approaches;Water pollution Predicting reductive transformation rates of halogenated aliph. compds. in water-sediment by different QSAR approaches.

Sato T, Watanabe K, Nagase H, Kito H, Niikawa M, Yoshioka Y. **Investigation of the hemolytic effects of various organophosphoric acid triesters (OPEs) and their structure-activity relationship**. Toxicol Environ Chem 1997;59(1-4):305-13.

Soskic M, Plavsic D, Trinajstic N. **Inhibition of the Hill reaction by 2-methylthio-4,6-bis (monoalkylamino)-1,3,5-triazines. A QSAR study**. Theochem 1997;394(1):57-65.

Tamaru M, Inoue J, Hanai R, Tachikawa S. **Studies of the new herbicide KIH-6127. 4. Crystal structure of KIH-6127 and quantitative structure-activity relationship of the iminoxy moiety of KIH-6127 derivatives**. J Agric Food Chem 1997;45(7):2777-83.

Taningher M, Malacarne D, Mancuso T, Peluso M, Pescarolo MP, Parodi S. **Methods for predicting carcinogenic hazards: new opportunities coming from recent developments in molecular oncology and SAR studies.** Mutat Res 1997;391(1-2):3-32.
BIOSIS COPYRIGHT: BIOL ABS. Without epidemiological evidence, and prior to either short-term tests of genotoxicity or long-term tests of carcinogenicity in rodents, an initial level of information about the carcinogenic hazard of a chemical that perhaps has been designed on paper, but never synthesized, can be provided by structure-activity relationship (SAR) studies. Herein, we have reviewed the interesting strategies developed by human experts and/or computerized approaches for the identification of structural alerts that can denote the possible presence of a carcinogenic hazard in a novel molecule. At a higher level of information, immediately below epidemiological evidence, we have discussed carcinogenicity experiments performed in new types of genetically engineered small rodents. If a dominant oncogene is already mutated, or if an allele of a recessive oncogene is inactivated, we have a model animal with (n-1) stages in the process of carcinogenesis. Both genotoxic and receptor-mediated carcinogens can induce cancers in 20-40% of the time required for classical murine strains. We have described the first interesting results obtained using these new artificial animal models for carcinogenicity studies. We have also briefly discussed other types of engineered mice (lac operon transgenic mice) that are especially suitable for detecting mutagenic effects in a broad spectrum of organs and tissues and that can help to establish mechanistic correlations between mutations and cancer frequencies in specific target organs. Finally, we have reviewed two complementary methods that, while obviously also feasible in rodents, are especially suitable for biomonitoring studies. We have illustrated some of the advantages and drawbacks related to the detection of DNA adducts in target and surrogate tissues using the 32P-DNA postlabeling technique, and we have discussed the possibility of biomonitoring mutations in different human target organs using a molecular technique that combines the activity of restriction enzymes with polymerase chain reaction (RFLP/PCR). Prediction of carcinogenic hazard and biomonitoring are very wide-ranging areas of investigation. We have therefore selected five different subfields for which we felt that interesting innovations have been introduced in the last few years. We have made no attempt to systematically cover the entire area: such an endeavor would have produced a book instead of a review article.

Todeschini R, Gramatica P. **3D-modeling and prediction by WHIM descriptors. Part 6. Application of WHIM descriptors in QSAR studies**. Quant Struct Act Relat 1997;16(2):120-5.

CBAC COPYRIGHT: CHEM ABS Three-dimensional mol. indexes (WHIM descriptors) are used to search for quant. structure-activity relationships to investigate the physico-chem. properties and biol. activities of different classes of environmental important compds. Chlorobenzenes are studied for their interesting physico-chem. properties, e.g. melting and b.ps., soly., lipophilicity (log Kow), bioconcn. factor (BCF), and for toxicity (Micro-tex test and algae). The antagonism of N,N-dimethyl-2-halo-phenethylamines to epinephrine and histamine is successfully modeled and compared with other models in the literature. Finally, good QSAR models are obtained for modeling the receptor binding affinities (RB) and inductions of aryl hydrocarbon hydroxylase (AHH) for some dioxin analog compds., polyhalogenated aryl derivs. All the obtained models confirm the high modeling power of the WHIM descriptors.

Tokarski JS, Hopfinger AJ. **Prediction of ligand-receptor binding thermodynamics by free energy force field (FEFF) 3D-QSAR analysis: application to a set of peptidometic renin inhibitors**. J Chem Inf Comput Sci 1997;37(4):792-811.
CBAC COPYRIGHT: CHEM ABS A methodol. is presented and applied in which the accurate estn. of ligand-receptor binding thermodn. is achieved by formulating the calcn. as a QSAR problem. When the receptor geometry is known, the free energy force field (FEFF) ligand-receptor binding energy terms are be calcd. and used as independent variables in constructing FEFF 3D-QSARs. The FEFF 3D-QSAR anal. of a series of transition state inhibitors of renin was carried out. From a statistical anal. of the free energy contributions to the binding process, FEFF 3D-QSARs were constructed that reveal the change in solvation free energy upon binding and the intramol. vacuum internal energy of the ligand in the unbound state and are the most significant FEFF terms in detg. the binding free energy, DELTAG. Other terms, such as ligand stretching, bending, and torsion energy changes, the intermol. van der Waals interaction energy, and change in ligand conformational entropy upon binding, are also found to make significant contributions in some FEFF 3D-QSAR DELTAG models and in DELTAH and DELTAS binding models. Overall, a relatively small no. of the thermodn. contributions to the ligand-receptor binding process dominates the thermodn. of binding in a given model.

Tronchet J, Grigorov M, Dolatshahi N, Moriaud F, Weber J. **A QSAR study confirming the heterogeneity of the HEPT derivative series regarding their interaction with HIV reverse transcriptase**. Eur J Med Chem 1997;32(4):279-99.
CBAC COPYRIGHT: CHEM ABS QSAR concerning the anti-HIV and cytotoxic activities of a series of HEPT analogs has been established using a Hansch-type approach (TSAR), a neural network approach (TSAR) and a pharmacophore search method (CATALYST). The techniques employed allowed reliable activity predictions and confirmed the heterogeneity of this series of compds., which was previously established in biochem. expts.

Turkov M, Rashi S, Noam Z, Gordon D, Ben Khalifa R, Stankiewicz M, Pelhate M, Gurevitz M. **In vitro folding and functional analysis of an anti-insect selective scorpion depressant neurotoxin produced in Escherichia coli.** Protein Expr Purif 1997;10(1):123-31.
The selective toxicity of depressant scorpion neurotoxins to insects is useful in studying insect sodium channel gating and has an applied potential. In order to establish a genetic system enabling a structure-activity approach, the functional expression of such polypeptides is required. By engineering the cDNA

encoding the depressant scorpion neurotoxin, LahIT2, behind the T7 promoter, large amounts of recombinant insoluble and nonactive toxin were obtained in Escherichia coli. Following denaturation and reduction, the recombinant protein, constructed with an additional N-terminal methionine residue, was subjected to renaturation. Optimal conditions for reconstitution of a functional toxin, having a dominant fold over many other possible isoforms, were established. The recombinant active toxin was purified by RP-HPLC and characterized. Toxicity (ED50) to insects, binding affinity (IC50) to an insect receptor site, and electrophysiological effect on an insect axonal preparation were found to be similar to those of the native toxin. Substitution of the C-terminal glycine by a Gly-Lys-Lys triplet did not abolish folding but affected toxicity (3.5-fold decrease) of LqhIT2. Apparently, this efficient bacterial expression system (500 micrograms HPLC-purified toxin/1 liter E. coli culture) provides the means for studying structure/ activity relationship and the molecular basis for the phylogenetic selectivity of scorpion depressant neurotoxins.

Wang H, Wang Z, Chen K, Ji R. **Quantum chemistry and QSAR studies of optical isomers of 3-methylfentanyl and ohmefentanyl**. J Chin Pharm Sci 1996;5(3):113-20.
CBAC COPYRIGHT: CHEM ABS Quantum chem. and QSAR investigations were carried out to understand why a small structural change lead to the fact that analgesic activities of 4 3-methylfentanyl and 8 ohmefentanyl optical isomers were highly different. The spatial relationship between 3-Me and the anilidophenyl group was most important. Other factors, such as the net at. charge on carbonyl oxygen and the configuration of 1-beta-hydroxy group, influence the pharmacodynamic action, too. Partial least square method was used for the construction of QSAR model, and the use of 3 latent variables obtained quite satisfactory correlation and predictive ability.

Wang M, Huang N, Yang G, Guo Z. **Study on 3D-QSAR of retinoids - 3D-interaction between retinoids and their receptor**. J Chin Pharm Sci 1996;5(2):57-62.
CBAC COPYRIGHT: CHEM ABS Epididymal retinoic acid binding protein (ERABP) was used as a template to simulate the interaction between 18 retinoids and their receptor. The DOCK program was used to deduce an equation to predict the drug-receptor binding const. The mol. conformers after docking were used in CoMFA anal. to obtain a model of this series of compds. The results suggest that the model resulted from the CoMFA anal. using the conformers after DOCK reduced the difficulty and ambiguity in conformation optimization and alignment.

Yu Q, Zhu L, Cai G, Wu N. **[CNDO/2 study on quantitative structure-activity relationships at N1 for 1-substituted-7-[3-(ethylamino methyl)-1-pyrrolidinyl]-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolin- ecarboxylic acids]**. Zhongguo Yaowu Huaxue Zazhi 1995;5(1):23-7, 39. (Chi)
CBAC COPYRIGHT: CHEM ABS In this paper, we carried on a research on the active conformations of N1 position of 1-substituted-7-[3-[(ethylamino)methyl]-1-pyrrolidinyl]-6, 8-difluoro-1, 4-dihydro-4-oxo-3-quinolinecarboxylic acids by CNDO/2 and first reported the QSAR which contained FUMO, super-delocalizability, frontier electron d., L, B1 and B5 et al. Our results were better than that of Domagala. The equations indicated that the antibacterial potency was strongly dependent on STERIMOL length and width and fN of the N1 substituted and FUMO of the mol.

Zefirov NS, Palyulin VA, Radchenko EV. **[Molecular field topology analysis in quantitative**

structure-activity relationship studies of organic compounds]. Dokl Akad Nauk 1997;352(5):630-3. (Rus)
CBAC COPYRIGHT: CHEM ABS Method of mol. field topol. anal. based on construction of mol. supergraph and on anal. of local physicochem. parameters of structures is suggested for QSAR studies.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Amwayi PJ, Otiang'a-Owiti GE. **Use of biometric embryonic growth parameters as indicator of exposure to a teratogen.** East Afr Med J 1997;74(1):6-11.
BIOSIS COPYRIGHT: BIOL ABS. Disturbances in embryonic growth were studied in 233 foetuses harvested on day 14.5 of gestation, after the administration of various doses of 5 Fluoro-2' deoxyuridine (FUdR) to pregnant mice on day 11.0 of gestation. Measurements of crown-rump length (CRL) and mean wet body weights showed a significant retardation of embryonic growth ($p<0.001$), following doses of 30, 80 and 100 mg FUdR per kg maternal body weight. Compared to the controls, whole FUdR-treated embryos that had been macerated, cleared and double stained with alcian blue 8GX plus alizarin red S for skeletal anlage, showed that ossification had not commenced in the vertebral bones of tail. All bones in the craniofacial region and limbs including the girdles, were smaller, while there were distortions of the long bones. The severity of the changes were dependent on the concentration of FUdR dose administered. Among the live FUdR-treated foetuses harvested, 95% had mesomelic limb defects. The incidence of delay or prevention of palatal processes elevation was 79%, 49%, 21% and 30% respectively for 0 mg (control), 30 mg, 80 mg and 100 mg FUdR doses. The results show that administration of a teratogenic agent (FUdR) causes retardation of growth which correlates with abnormalities of the secondary palate and limbs. It is proposed that the initial screening of potential teratogenic substances in food, such as preservatives or colourings, may be carried out by monitoring changes in secondary palate and limb development, including biometric growth parameters of an animal model.

Anderson D, Dobrzynska MM, Basaran N. **Effect of various genotoxins and reproductive toxins in human lymphocytes and sperm in the comet assay**. Teratog Carcinog Mutagen 1997;17(1):29-43.
CBAC COPYRIGHT: CHEM ABS There have been conflicting reports as to whether the mean sperm count in some men has diminished over the last 50 yr. The downward trend has been suggested to coincide with an increase in exposure to estrogen-like compds. These estrogenic substances are ubiquitous in the environment. The authors have examd. the effect of such substances (diethylstilbestrol, beta-estradiol, daidzein, genistein, and nonylphenol) in the single cell gel electrophoresis assay (Comet assay) in human sperm and compared responses with those from human peripheral lymphocytes in the same donor and in peripheral lymphocytes from a female donor. In addn., effects from the estrogens have been compared to those from known reprotoxins and genotoxins. These include lead sulfate, nitrate and acetate, dibromochloropropane, ethylene glycol monoethyl ether, 1,2-epoxybutane, and 1,2,3,4-diepoxybutane. All compds. produced pos. responses, but ethylene glycol monoethyl ether only produced pos. responses in sperm cells in the male and not in peripheral lymphocytes, and similarly the phytoestrogens (genistein, daidzein) were less responsive in the peripheral lymphocytes in the male than in the sperm. This may be due to greater sensitivity of sperm cells because of their lack of repair. However, since damage was generally seen over a similar dose range, a one-to-one ratio of somatic and

germ cell damage was obsd. and has implications for human for risk assessment purposes.

Barabino SM, Spada F, Cotelli F, Boncinelli E. **Inactivation of the zebrafish homolog of Chx10 by antisense oligonucleotides causes eye malformations similar to the ocular retardation phenotype**. Mech Dev 1997;63(2):133-43.
CBAC COPYRIGHT: CHEM ABS We report the cloning of a zebrafish paired-type homeobox gene, Alx, closely related to the murine Chx10 and the goldfish Vsx-1 homeodomain proteins. Alx is first expressed at about 12 h post-fertilization (hpf) when optic vesicles appear. Its expression is restricted to the early retinal neuroepithelium, whereas no signal can be detected in the optic placode. Later, Alx expression follows the differentiation of the neural retina. Inhibition expts. with antisense oligonucleotides resulted in specific eye malformations which are reminiscent of the phenotype of ocular retardation (or) mice, caused by a spontaneous Chx10 mutation. The expression of other developmentally relevant genes such as pax(zf-a), pax(zf-b) and krx-20 was not affected in the antisense treated embryos.

Barton HA, Andersen ME. **Dose-response assessment strategies for endocrine-active compounds.** Regul Toxicol Pharmacol 1997;25(3):292-305.
Hazard identification provides evidence for the potential of compounds to cause effects in exposed people. Dose-response assessments define the range of exposure conditions associated with minimal risks of adverse effects. With endocrine-active compounds (EACs), the vast majority of resources are presently being applied to hazard identification. In the past, dose-response assessments have been based on empirical analysis of these relationships. The empirical underpinnings of these models do not permit conclusions about the low-dose and interspecies extrapolation of the animal study results. Biologically based dose-response assessments relying on knowledge of mode-of-action (pharmacodynamics) and dosimetry (pharmacokinetics) offer promise to develop broadly applicable strategies for quantitative dose-response assessments with these EACs. These approaches would focus on normal physiological endocrine signaling processes in the body, their associated control mechanisms, and the interaction among different internal signaling pathways. A critical element of signaling is regulation of the concentration of the signaling compound, e.g., steroid sex hormone. Exogenous compounds that act as signals but evade the normal homeostatic control of signaling compound concentrations represent one class of EACs. Other molecular components of these signaling systems include receptors, second messengers, and DNA-accessory/transcriptional protein complexes; EACs may interfere with the functions of any of these components. The challenge facing the toxicology and risk assessment professions is to base regulatory strategies on the interaction of these EACs with the fundamental control mechanisms which regulate responses throughout the body and to determine the extent to which these interactions create specific dose-response behaviors in the living animals.

Brockerhoff SE, Hurley JB, Niemi GA, Dowling JE. **A new form of inherited red-blindness identified in zebrafish.** J Neurosci 1997;17(11):4236-42.
A red-blind zebrafish mutant, partial optokinetic response b (pob), has been isolated by measuring eye movements of larvae in a three-generation screen for recessive mutations affecting the visual system. pob larvae exhibit eye movements in response to rotating black and white stripes illuminated with white light, but they do not move their eyes when the stripes are illuminated with red light. Physiological,

immunohistochemical, and in situ hybridization analyses of pob retinas showed a selective loss of red-sensitive cones at 5 days postfertilization (dpf). At 3 dpf, cells expressing red opsin are present, suggesting that red-sensitive cones form initially but then disappear rapidly.

Chimal-Monroy J, De Leon LD. **Differential effects of transforming growth factors beta1, beta2, beta3 and beta5 on chondrogenesis in mouse limb bud mesenchymal cells**. Int J Dev Biol 1997;41 (1):91-102.
CBAC COPYRIGHT: CHEM ABS The present study was performed to det. whether mammalian TGF-beta isoforms and Xenopus TGF-beta5 elicit a differential chondrogenic response on mesenchymal cells during mouse limb development. Results showed that TGF-beta isoforms produced a distinct chondrogenic pattern depending on embryonic stage. When they were applied to 5 day micromass cultures of limb mesenchymal cells from embryonic stages 19, 20 and 21, a differential response to all four TGF-beta isoforms assayed was obsd. By stage 19 the cells formed a uniform sheet of cartilage cells; by stage 20, mesenchymal cells were more responsive to TGF-beta1 and TGF-beta5 than at stages 19 and 21, showing an entire cell layer of chondrogenic cells with higher accumulation of extracellular matrix. The diminished effect of TGF-beta2 and TGF-beta3 at stages 20 and 21 was accompanied by a nodular pattern of chondrogenic cells rather than by a uniform sheet, as seen at stage 19. At stage 20 TGF-beta1 and TGF-beta5 enhanced the expression of sulfated proteoglycans, type II collagen, cartilage link protein and alk. phosphatase activity. In contrast, TGF-beta2 and TGF-beta3 caused less expression in the same parameters. Only a transient exposure to TGF-beta isoforms at days 1 and 2 of culture stimulate chondrogenesis, indicating that TGF-beta isoforms could regulate chondrogenesis at early stages of chondrocyte differentiation. However, when TGF-beta isoforms were applied to low d. cultures of mesenchymal cells, chondrogenesis was enhanced only by 25%, suggesting that TGF-beta isoforms enhanced cartilage differentiation to higher levels in micromass cultures than in situations in which little or no chondrogenic differentiation normally occurs.

Curtis SW, Shi H, Teng C, Korach KS. **Promoter and species specific differential estrogen-mediated gene transcription in the uterus and cultured cells using structurally altered agonists.** J Mol Endocrinol 1997;18(3):203-11.
Certain types of estrogenic compounds have been shown to have tissue-specific actions. In addition, some tissues may exhibit differential gene regulation by agonists and antagonists. Our previous studies using structurally modified estrogenic molecules had indicated differential effects on specific estrogen responses, indicating that the activity of the estrogen receptor protein can be altered depending not only upon the structure of the bound ligand but also the regulated gene itself. The mechanism of differential induction, however, was not determined, and might involve altered binding to the estrogen response element (ERE), altered transcription, or post-transcriptional modification of gene products. Our previous studies indicated that differential induction by modified diethylstilbestrol (DES) agonists could not be accounted for by differences in ligand affinity for the estrogen receptor (ER) or differential binding of the ER to a consensus vitellogenin A2 (vit A2) ERE. To determine if this differential hormonal responsiveness was reflected at the level of transcription, we analyzed mouse uterine mRNA of several estrogen-responsive genes, including glucose-6-phosphate dehydrogenase (G6PD), ornithine decarboxylase (ODC) and lactoferrin, by Northern blot following injection with the modified agonists DES, indenestrol A (IA), indenestrol B (IB) and Z-pseudo DES (ZPD). All compounds induced the

G6PD message, although IB and ZPD induced expression only transiently, while DES and IA maintained the message for 24 h. No difference in induction was seen for ODC message, which was induced equally by all the compounds. In contrast, lactoferrin, a highly estrogen-responsive gene, was induced only by DES and IA and not by the other agonists IB or ZPD, showing that the lactoferrin gene was differentially regulated by these compounds. To determine whether this difference was due to altered transcriptional activity, the mouse lactoferrin estrogen-responsive module (mERM) linked to a chloramphenicol acetyl transferase (CAT) reporter gene was tested in transfected cells. Using the mouse estrogen receptor in RL95 cells, DES and IA induced expression of CAT, but IB did not, confirming the differential response seen in vivo. To show whether this difference in transcription occurred because of altered binding to the lactoferrin ERE, which is not a perfect consensus ERE a gel shift assay was used to examine DNA binding of ER bound to the agonists. All ligands produced equivalent binding to the lactoferrin ERE suggesting that differential regulation was not a result of altered DNA binding. Taken together, these observations indicate that the differential induction of lactoferrin by these compounds occurs via altered activation of the transcriptional components unique to lactoferrin and is likely to involve altered interaction with co-activators. Surprisingly, unlike the mouse ER, the human estrogen receptor activated and induced expression of lactoferrin estrogen-responsive module-CAT with all the compounds. Mouse ER is also known to vary from the human ER in its activity with the triphenylethylene estrogen tamoxifen, which has agonist activity with the mouse ER but mixed antagonist/agonist activity with the human ER. The data show that human and mouse estrogen receptors are activated differently by this group of stilbestrol estrogen ligands when assayed on the lactoferrin response element, which is the first description of this type of gene and species specific difference. Lactoferrin gene regulation by estrogen receptor can be used as a model to study the mechanism of differential gene activation by different estrogen agonists and antagonists using a more physiological situation than commonly used with in vitro gene reporter systems.

Daston GP. **Advances in understanding mechanisms of toxicity and implications for risk assessment.** Reprod Toxicol 1997;11(2-3):389-96.
BIOSIS COPYRIGHT: BIOL ABS. Knowledge of mechanism of action of a toxicant can greatly improve the accuracy of risk estimation by replacing with data the many default assumptions of risk assessment. Results from studies on comparative pharmacokinetics, metabolism, cell biology, and molecular biology have been successfully applied to problems of interspecies extrapolation, interindividual differences in susceptibility, and the relevance of high-dose findings for low-dose risk estimation. Examples are provided. Extremely rapid progress in understanding the molecular control of embryonic pattern formation and organogenesis has the potential to significantly improve the accuracy of risk assessment, especially by providing a sounder basis for characterizing interspecies differences, individual susceptibility, and multifactorial (gene-environment) etiologies of abnormal development. However, it will be necessary to quantitate toxicant-induced changes at the molecular level and to determine the level of change needed to perturb higher levels of biological organization at which adverse effects are manifested. It will also be important for risk assessment methodology to evolve so that it can better and more routinely accommodate mechanistic information. There is great potential for the recent and coming advances in knowledge of the molecular and cellular basis of abnormal development to be applied to risk assessment. Consideration should be given to shifting some of the resources now allocated to hazard screening to investigating the mechanisms of chemically induced abnormal

development.

Diehl SR, Erickson RP. **Genome scan for teratogen-induced clefting susceptibility loci in the mouse: evidence of both allelic and locus heterogeneity distinguishing cleft lip and cleft palate.** Proc Natl Acad Sci U S A 1997;94(10):5231-6.
BIOSIS COPYRIGHT: BIOL ABS. Nonsyndromic clefting of the lip and palate in humans has a highly complex etiology, with both multiple genetic loci and exposure to teratogens influencing susceptibility. Previous studies using mouse models have examined only very small portions of the genome. Here we report the findings of a genome-wide search for susceptibility genes for teratogen-induced clefting in the AXB and BXA set of recombinant inbred mouse strains. We compare results obtained using phenytoin (which induces cleft lip) and 6-aminonicotinamide (which induces cleft palate). We use a new statistical approach based on logistic regression suitable for these categorical data to identify several chromosomal regions as possible locations of clefting susceptibility loci, and we review candidate genes located within each region. Because cleft lip and cleft palate do not frequently co-aggregate in human families and because these structures arise semi-independently during development, these disorders are usually considered to be distinct in etiology. Our data, however, implicate several of the same chromosomal regions for both forms of clefting when teratogen-induced. Furthermore, different parental strain alleles are usually associated with clefting of the lip versus that of the palate (i.e., allelic heterogeneity). Because several other chromosomal regions are associated with only one form of clefting, locus heterogeneity also appears to be involved. Our findings in this mouse model suggest several priority areas for evaluation in human epidemiological studies.

Ensenbach U, Nagel R. **Toxicity of binary chemical mixtures: effects on reproduction of zebrafish (Brachydanio rerio).** Arch Environ ContamToxicol 1997;32(2):204-10.
BIOSIS COPYRIGHT: BIOL ABS. A complete life-cycle test with zebrafish was carried out with different concentrations of the binary mixture 3,4-dichloroaniline and lindane under flow-through conditions. Length and weight of fish of the F1-generation were reduced, even in the lowest test concentration of 2 mug/L 3,4-dichloroaniline and 40 mug/L lindane. The same effects were found in the early life stage test for the F2-generation. In the mixture of 100 mug/L 3,4-dichloroaniline and 40 mug/L lindane, fish which were exposed for their whole life time stopped spawning, irreversibly; the fish population will become extinct. In an additional experiment, fish were exposed to the same xenobiotic concentrations after reaching maturity. In this case, egg production was reduced. Cessation of egg production occurs in a concentration of 200 mug/L 3,4-dichloroaniline and 40 mug/L lindane. Nevertheless, effects on spawning are influenced by duration of exposure and the life stages of exposure.

Fort DJ, Propst TL, Stover EL. **Evaluation of the developmental toxicity of 4-bromobenzene using frog embryo teratogenesis assay-Xenopus: possible mechanisms of action.** Teratogen Carcinogen Mutagen 1996;16(6):307-15.
BIOSIS COPYRIGHT: BIOL ABS. Potential mechanisms of 4-bromobenzene-induced developmental toxicity were evaluated using frog embryo teratogenesis assay-Xenopus (FETAX). Early X. laevis embryos were exposed to 4-bromobenzene in two separate definitive concentration-response tests with and without an exogenous metabolic activation system (MAS) or selectively inhibited MAS. The MAS was treated with carbon monoxide (CO) to modulate P-450 activity, cyclohexene oxide (CHO) to

modulate epoxide hydrolase activity, and diethyl maleate (DM) to modulate glutathione conjugation. Addition of the intact MAS, and particularly the CHO- and DM-inhibited MASs, dramatically increased the embryo lethal potential of 4-bromobenzene. Addition of the CO-inhibited MAS decreased the developmental toxicity of activated 4-bromobenzene to levels approximating that of the parent compound. Results from these studies suggested that a highly toxic arene oxide intermediate of 4-bromobenzene formed as the result of mixed function oxidase (MFO)-mediated metabolism may play an important role in the developmental toxicity of 4-bromobenzene in vitro. Furthermore, both epoxide hydrolase and glutathione conjugation appeared to be responsible for activated 4-bromobenzene detoxification.

Ghanooni M, Mattison DR, Zhang YP, Macina OT, Rosenkranz HS, Klopman G. **Structural determinants associated with risk of human developmental toxicity**. Am J Obstet Gynecol 1997;176 (4):799-806.

Hagedorn M, Kleinhans FW, Wildt DE, Rall WF. **Chill sensitivity and cryoprotectant permeability of dechorionated zebrafish embryos, Brachydanio rerio.** Cryobiology 1997;34(3):251-63.
The zebrafish (Brachydanio rerio) was used as a model for basic studies of the chilling sensitivity, permeability and toxicity of cryoprotectants. In both intact and dechorionated embryos, early-stage embryos (1.25, 1.5, 1.75, and 2 h) were more susceptible ($P < 0.05$) to chilling injury at 0 degrees C than late-stage embryos (50, 75, and 100% epiboly and three-somite stage). Moreover, enzymatic removal of the chorion did not alter ($P > 0.05$) this pattern of sensitivity to chilling. Eight-hour zebrafish embryos tolerated short-term exposures to temperatures ranging from 4 to 23 degrees C for 3.5 h with no detrimental developmental effects. The permeability of dechorionated embryos to cryoprotectants was examined by measuring the kinetics of volumetric change at various developmental stages (16 cells to six somites or ca. 1.25 to 14 h postfertilization) at 28.5 degrees C. The dechorionated zebrafish embryo is composed of two complex cellular compartments (i.e., a large yolk and the developing blastoderm). From 40 to 100% epiboly, the volumes of yolk and blastoderm remained constant, ca. 82 and 18%, respectively. However, these volumes changed rapidly after epiboly. For example, at the six-somite stage, the yolk composed 61% of the total volume, whereas the blastoderm composed 39%. When three- and six-somite embryos were placed in 1.5 and 2.0 M cryoprotectants (dimethyl sulfoxide and propylene glycol), osmometric measurement of volume changes indicated no permeation of the cryoprotectants. However, some permeation was observed for six-somite embryos immersed in a 2.0 M methanol solution, but not for 3-somite embryos. For up to 30 min at room temperature, these cryoprotectant solutions were toxic to zebrafish embryos; however, 1.5 M glycerol and ethylene glycol solutions were. We conclude that the complex nature of the zebrafish embryo reduces the effectiveness and predictive value of light microscopical measurements for cryoprotectant permeability studies.

Kapron CM, Trasler DG. **Genetic determinants of teratogen-induced abnormal development in mouse and rat embryos in vitro.** Int J Devel Biol 1997;41(2):337-44.
BIOSIS COPYRIGHT: BIOL ABS. The response of an embryo to a teratogenic treatment is often critically dependent on its genetic makeup. However, in conventional in vivo studies of gene-teratogen interactions it may be difficult to distinguish between the effects of genes that are carried by the embryo and those that are carried by the mother. It is likewise not easy to determine whether an observed

interaction is between a particular gene and the parent compound administered, or whether it is with a metabolite that has been generated by the maternal system. The use of whole rodent embryo culture offers certain advantages in the study of gene-teratogen interactions. Not only can the effects of metabolism and the maternal genotype be more carefully controlled, but the stage of development at which embryos of different genotypes are exposed can be matched. Rodent whole embryo culture has been used to a limited extent to study interactions between single gene mutations and teratogenic treatments, variations in responses of different strains to teratogens, as well as species differences in response to teratogens. These studies point to the need to precisely control the stage of development at the time of treatment in order to be able to make valid comparisons. But, even more important, they highlight the versatility of the whole embryo culture technique, and underscore the need for its wider use in evaluating the relative contribution of genes and environment to abnormal embryonic development.

Kavlock RJ. **Recent advances in mathematical modeling of developmental abnormalities using mechanistic information.** Reprod Toxicol 1997;11(2-3):423-34.
BIOSIS COPYRIGHT: BIOL ABS. During the last several years, significant changes in the risk assessment process for developmental toxicity of environmental contaminants have begun to emerge. The first of these changes is the development and beginning use of statistically based dose-response models (the benchmark dose (BMD) approach) that better utilize data derived from existing testing approaches. Accompanying this change is the greater emphasis placed on understanding and using mechanistic information to yield more accurate, reliable, and less uncertain risk assessments. The next stage in the evolution of risk assessment will be the use of biologically based dose-response (BBDR) models that begin to build into the statistically based models factors related to the underlying kinetic, biochemical, and/or physiologic processes perturbed by a toxicant. Such models are now emerging from several research laboratories. The introduction of quantitative models and the incorporation of biologic information into them has pointed to the need for even more sophisticated modifications for which we offer the term embryologically based dose-response (EBDR) models. Because these models would be based upon the understanding of normal morphogenesis, they represent a quantum leap in our thinking, but their complexity presents daunting challenges both to the developmental biologist and the developmental toxicologist. Implementation of these models will require extensive communication between developmental toxicologists, molecular embryologists, and biomathematicians. The remarkable progress in the understanding of mammalian embryonic development at the molecular level that has occurred over the last decade combined with advances in computing power and computational models should eventually enable these as yet hypothetical models to be brought into use.

Klinefelter GR, Laskey JW, Ferrell J, Suarez JD, Roberts NL. **Discriminant analysis indicates a single sperm protein (SP22) is predictive of fertility following exposure to epididymal toxicants.** J Androl 1997;18(2):139-50.
In a previous study, we found that ethane dimethanesulphonate (EDS) compromised the fertilizing ability of proximal cauda epididymal sperm from the rat within 4 days of exposure, an effect that persisted in castrated, testosterone (T)-implanted animals, establishing direct action on the epididymis. This EDS-induced reduction in fertilizing ability was highly correlated with a quantitative decrease in specific sperm protein. Here we sought to determine whether the fertility of proximal cauda epididymal sperm recovered from animals exposed to a variety of male reproductive toxicants could be predicted by

assessing quantitative changes in specific sperm protein(s), or whether more common endpoints (e.g., sperm motility, sperm morphology, serum and epididymal tissue T, cauda epididymal sperm reserves) also are required to predict fertility. Intact adult male rats were dosed with EDS (25 or 50 mg/kg), chloroethylmethanesulphonate (CEMS; 12.5 or 18.75 mg/kg), or epichlorohydrin (EPI; 3 or 6 mg/kg) daily for 4 days. Castrated, T-implanted rats were dosed with hydroxyflutamide (HFLUT; 12.5 or 25 mg/kg) daily for 5 days. On day 5, proximal cauda epididymal sperm were inseminated in utero into receptive, cervically stimulated adult females, and on day 9, fertility (implants/corpora lutea) was assessed. Fertility-was decreased by the higher dose of each toxicant ($P < 0.05$) and also by the lower dose of EPI and HFLUT. Likewise, an acidic 22 kDa sperm protein (SP22) was decreased quantitatively ($P < 0.05$) in silver-stained two-dimensional gels by the higher dose of each toxicant as well as by the lower dose of EPI and HFLUT. Although sperm motility and serum T were altered by specific exposures, these endpoints were not useful in predicting fertility. In contrast, SP22 was highly correlated ($P < 0.0001$; $r2 = 0.83$) with fertility. Indeed, the amount of SP22 correctly predicted 90% and 94% of the fertile (> 50% fertility) and subfertile (< 50 fertility) animals, respectively, when discriminant analysis was performed. Thus, the amount of SP22 in a cauda epididymal sperm sample may be a useful predictor of fertility in toxicant-treated animals.

Kononen DW, Gorski RA. **A method for evaluating the toxicity of industrial solvent mixtures**. Environ Toxicol Chem 1997;16(5):968-76.
CBAC COPYRIGHT: CHEM ABS The toxicity of a mixt. of toluene and mixed xylenes was studied with the frog embryo teratogenesis assay-Xenopus (FETAX). Single compd. expts. with either toluene or mixed xylenes show that growth inhibition, not mortality or malformation, is the most sensitive toxic endpoint in the FETAX system (i.e., growth is inhibited at lower exposure concns. than those resulting in malformations and mortality). A multiple logistic regression anal. model described mortality and malformation as functions of exposure concns. of toluene and mixed xylenes. The results indicate that toluene and mixed xylenes are additive in their effects on mortality in the FETAX system. Further experimentation is necessary to validate the model's ability to describe malformation as a function of coexposure to toluene and mixed xylenes.

Liu D, Le Drean Y, Ekker M, Xiong F, Hew CL. **Teleost FTZ-F1 homolog and its splicing variant determine the expression of the salmon gonadotropin IIbeta subunit gene**. Mol Endocrinol 1997;11(7):877-90.
CBAC COPYRIGHT: CHEM ABS Steroidogenic factor 1, a member of the fushi tarazu factor 1 (FTZ-F1) subfamily of nuclear receptors, is a key regulator in mammalian reprodn. From an embryonic cDNA library, the zebrafish homolog of FTZ-F1 (zFF1A) and an alternatively spliced variant (zFF1B) were isolated. ZFF1B represented a C-terminally truncated version of zFF1A. Whole mount in situ hybridization and reverse transcriptase-PCR anal. revealed that both zFF1A and B transcripts were present in the developing pituitaries, adult fish brain, gonads, and liver.

Mizell M, Romig ES. **The aquatic vertebrate embryo as a sentinel for toxins: zebrafish embryo dechorionation and perivitelline space microinjection.** Int J Devel Biol 1997;41(2):411-23.
BIOSIS COPYRIGHT: BIOL ABS. Pollution of aquatic ecosystems poses a serious threat to aquatic organisms and ultimately the entire ecosystem. Understanding how a toxin affects embryonic

development is key to determining the risk a pollutant represents to the environment. Extraembryonic membranes, such as the chorion of fish eggs, provide a protective barrier between the embryo and the environment. Although the fish chorion excludes many chemical pollutants, some noxious agents can still gain access to the aquatic embryo. Therefore a monitoring system that tests the effects directly upon the embryo must be established. Although exposure to a single toxin in the laboratory can determine the concentration at which a pollutant becomes a health or environmental hazard, embryos and adults in nature are not merely affected by a single chemical, but are exposed to mixtures of different pollutants. Zebrafish (Danio rerio) and medaka (Oryzias latipes) embryos were employed for the rapid observation of the effects of single chemicals and chemical mixtures on development. Using dechorionation and a perivitelline space microinjection system, the embryos were effective sentinels for low concentrations of aquatic pollutants. The developmental effects of small quantities of toxins were observed. Embryos treated during the late gastrula stage of development with hexachlorobenzene (HCB); 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD); toluene; benzene; or mixtures of these chemicals developed cardiovascular abnormalities. The zebrafish dechorionation exposure technique, Micro Intrachorionic Zebrafish Embryo Live Laboratory test, was especially effective in testing the pollutant mixtures. Combinations of both TCDD and benzene (as well as the toluene and benzene combinations) were tested and the mixtures acted synergistically; the combinations were more toxic than either chemical by itself. Hexachlorobenzene- and TCDD-treated embryos tested positively for expression of cytochrome P450 1A indicating that the cytochrome metabolic pathways were already functional in these early embryos, and suggested that a product of the cytochrome system may be involved in HCB and TCDD pollution associated cardiovascular defects.

Muller F, Williams DW, Kobolak J, Gauvry L, Goldspink G, Orban L, MaClean N. **Activator effect of coinjected enhancers on the muscle-specific expression of promoters in zebrafish embryos**. Mol Reprod Dev 1997;47(4):404-12.

Propst TL, Fort DJ, Stover EL, Schrok B, Bantle JA. **Evaluation of the developmental toxicity of benzo(a)pyrene and 2-acetylaminofluorene using Xenopus: modes of biotransformation. Stover Group.** Drug Chem Toxicol 1997;20(1-2):45-61.
BIOSIS COPYRIGHT: BIOL ABS. The developmental toxicities of benzo(a)pyrene (BAP) and 2-acetylaminofluorene (AAF) were evaluated using FETAX (Frog Embryo Teratogenesis Assay - Xenopus). Young X. laevis embryos were exposed to these two compounds in each of two separate concentration-response experiments with and without an exogenous metabolic activation system (MAS) and/or inhibited MAS. The MAS was treated with cimetidine (CIM), ellipticine (ELL), or alpha-naphthoflavone (alpha-N) to selectively modulate cytochrome P-450 activity. Bioactivation of both of these compounds was indicated by increased developmental toxicity observed in MAS tests. Results obtained in treated MAS tests indicated that BAP was predominantly activated by Cytochrome P-450 isozyme CYP1A1. AAF bioactivation was shown to be only partly mediated by CYP1A1/2. Detoxification pathways for these two compounds were investigated by treatment of the MAS with cyclohexene oxide (CHO) and diethyl maleate (DM) to inhibit the epoxide hydroxylase and glutathione conjugation pathways, respectively. Results indicated that epoxide hydroxylase was primarily responsible for the detoxification of BAP, with glutathione conjugation playing a secondary role. Detoxification of AAF by these two pathways was not indicated.

Randerath K, Zhou GD, Monk SA, Randerath E. **Enhanced levels in neonatal rat liver of 7,8-dihydro-8-oxo-2'-deoxyguanosine (8-hydroxydeoxyguanosine), a major mutagenic oxidative DNA lesion.** Carcinogen 1997;18(7):1419-21.
The purpose of this study was to determine whether the level of 7,8-dihydro-8-oxo-2'-deoxyguanosine (8-hydroxy-2'-deoxyguanosine) (8-oxo-dG), a major mutagenic DNA oxidation product, is enhanced in newborn rat liver DNA as a consequence of oxidative stress incurred during the early postnatal period. 32P-postlabeling showed this adduct to increase approximately 2-fold from the 20th day of gestation (2 days before birth) to a peak level at 50-53 h after birth. Postnatal levels exceeded fetal levels at all time points investigated, i.e. 0.5-1, 8, 24, 50-53, 100, 216 and 432 h after birth. Increased formation of this mutagenic DNA lesion during the critical postnatal phase when there is rapid cell proliferation in all tissues is proposed to contribute to carcinogenesis in susceptible tissues later in life.

Rudel R. **Predicting health effects of exposures to compounds with estrogenic activity: methodological issues.** Environ Health Perspect 1997;105(Suppl 3):655-63.
BIOSIS COPYRIGHT: BIOL ABS. Many substances are active in in vitro tests for estrogenic activity, but data from multigenerational and other toxicity studies are not available for many of those substances. Controversy has arisen, therefore, concerning the likelihood of adverse health effects. Based on a toxic equivalence factor risk assessment approach, some researchers have concluded that exposure to environmental estrogens is not associated with estrogen receptor (ER)-mediated health effects. Their rationale cites the low potency of these compounds in in vitro assays relative to estradiol, and the widespread exposure to pharmaceutical, endogenous, and dietary estrogens. This reasoning relies on two assumptions: that the relative estrogenic potency in in vitro assays is predictive of the relative potency for the most sensitive in vivo estrogenic effect; and that all estrogens act via the same mechanism to produce the most sensitive in vivo estrogenic effect. Experimental data reviewed here suggest that these assumptions may be inappropriate because diversity in both mechanism and effect exists for estrogenic compounds. Examples include variations in ER-ligand binding to estrogen response elements, time course of nuclear ER accumulation, patterns of gene activation, and other mechanistic characteristics that are not reflected in many in vitro assays, but may have significance for ER-mediated in vivo effects. In light of these data, this report identifies emerging methodological issues in risk assessment for estrogenic compounds: the need to address differences in in vivo end points of concern and the associated mechanisms; pharmacokinetics; the crucial role of timing and duration of exposure; interactions; and non-ER-mediated activities of estrogenic compounds.

Sakal E, Bignon C, Grosclaude J, Kantor A, Shapira R, Leibovitch H, Helman D, Nespoulous C, Shamay A, et al. **Large-scale preparation and characterization of recombinant ovine placental lactogen**. J Endocrinol 1997;152(2):317-27.
CBAC COPYRIGHT: CHEM ABS To clone ovine placental lactogen (oPL) cDNA, total RNA from sheep placental cotyledon was reverse transcribed and the single-stranded cDNA was PCR-amplified with 5' and 3' primers contg., resp., NcoI and PstI sites. The oPL cDNA fragment amplified between these two primers extended from A(-1) to the natural stop codon. The PCR product was gel-purified and subcloned into a Puc vector and the insert was sequenced on both stranded, revealing several differences relative to the published sequence: S19N, S69N, D129E and R165Q. We assume that these differences

can be accounted for by the high level of individual polymorphism, which has been described in detail for PLs of different species. The insert was subcloned into NcoI/PstI-digested pTrc99A procaryotic expression plasmid and protein expression was induced by isopropyl-1-thio-beta-D-galactopyranoside. Because of low expression, oPL's cDNA was further subcloned into pET8 procaryotic expression plasmid. Its expression in Escherichia coli strain BL21 transformed with this vector yielded 30-40 mg/L. The expressed protein, found in the inclusion bodies, was refolded into a monomer and purified on a Q-Sepharose column to homogeneity. Structural anal. using CD revealed a spectrum similar to that of human GH (hGH) thereby indicating proper refolding. Gel filtration and binding expts., including real-time kinetic measurements using the surface plasmon resonance method revealed that oPL forms transient homodimeric complexes with extracellular domains of prolactin receptors from rabbit, rat and bovine and with hGH receptor. The purified oPL was biol. active in an Nb2-11C cell proliferation bioassay, in its ability to stimulate beta-casein synthesis in explants of ovine and rabbit mammary gland and fat synthesis in explants of bovine mammary gland, and in a proliferation assay using FDC-P1 cells transfected with rabbit or hGH receptors.

Tyler CR, Van Der Eerden B, Jobling S, Panter G, Sumpter JP. **Measurement of vitellogenin, a biomarker for exposure to estrogenic chemicals, in a wide variety of cyprinid fish**. J Comp Physiol B 1996;166(7):418-26.
CBAC COPYRIGHT: CHEM ABS A carp (Cyprinus carpio) vitellogenin (VTG) RIA was tested for VTG of 9 species of fish. VTG from cyprinids showed good cross-reactivity in the following fish: bream (Abramis brama), roach (Rutilus rutilus), rudd (Scardinius erythropthalmus), gudgeon (Gobio gobio), minnow (Phoxinus phoxinus), fathead minnow (Pimephales promelas), zebrafish (Brachydanio rerio), and goldfish (Carassius auratus). The concns. of VTG in mature females were between a few 100 and 1000 mug/mL. Concns. of plasma VTG in immature females and in males were > 200 and < 20 ng/mL, resp.

Watanabe T, Iwase T. **Developmental and dysmorphogenic effects of glufosinate ammonium on mouse embryos in culture**. Teratog Carcinog Mutagen 1997;16(6):287-99.

Wine RN, Chapin RE. **Evaluation of the binding patterns of eleven FITC-conjugated lectins in Fischer 344 rat testes.** J Androl 1997;18(1):71-9.
The binding patterns of 11 recently commercially available fluorescein isothiocyanate-conjugated lectins that have been uncharacterized or undercharacterized in rat testes and/or have an unknown or complex carbohydrate specificity were evaluated in paraffin sections from Fischer 344 rat testes. Several of the lectins exhibited unique binding patterns that provide information about changes in carbohydrate domains, particularly during germ-cell maturation, that occur during spermatogenesis. Agaricus bisporus (ABA) lectin produced the most striking staining pattern in the cytoplasm of maturing germ cells, increasing in intensity until spermatid elongation, while the nuclei remained negative. In contrast, Cicer arietinum (CPA) strongly stained the nucleus of early leptotene/zygotene.

Winnier GE, Hargett L, Hogan BL. **The winged helix transcription factor MFH1 is required for proliferation and patterning of paraxial mesoderm in the mouse embryo**. Genes Dev 1997;11 (7):926-40.

CBAC COPYRIGHT: CHEM ABS The gene mfh1, encoding a winged helix/forkhead domain transcription factor, is expressed in a dynamic pattern in paraxial and persomitic mesoderm and developing somites during mouse embryogenesis. Expression later becomes restricted to condensing mesenchyme of the vertebrae, head, limbs, and kidney. A targeted disruption of the gene was generated by homologous recombination in embryonic stem cells. Most homozygous mfh1 null embryos die prenatally but some survive to birth, with multiple craniofacial and vertebral column defects. Using mol. markers, we show that the initial formation and patterning of somites occurs normally in mutants. Differentiation of sclerotome-derived cells also appears unaffected, although a redn. of the level of some markers [e.g., mtwist, mf1, scleraxis, and alpha1(II) collagen] is seen in the anterior of homozygous mutants. The most significant difference, however, is a marked redn. in the proliferation of sclerotome-derived cells, as judged by BrdU incorporation. This proliferation defect was also seen in micromass cultures of somite-derived cells treated with transforming growth factor.

MISCELLANEOUS

Bechgaard E, Bindseil E, Bagger M, Nielsen HW. **Reversibility and clinical relevance of morphological changes after nasal application of ephedrine nasal drops 1%**. Int J Pharm 1997;152 (1):67-73.
CAC COPYRIGHT: CHEM ABS To predict the toxicity of nasal formulations, various in vitro and in vivo techniques have been.

Backenbury TD, Appleton CC, Thurman G. **Mammal toxicity assessment of the plant molluscicide, Apodytes dimidiata (Icacinaceae), in South Africa.** Acta Tropica 1997;65(3):155-162.
BOSIS COPYRIGHT: BIOL ABS. Apodytes dimidiata has recently come to the fore as a potential plant molluscicide for schistosomiasis control in rural communities in South Africa. Prior to field applications of its leaves and extract to waterbodies, selected acute and sub-acute mammal toxicity tests were conducted in accordance with the Organisation of Economic Cooperation and Development (OECD) Guidelines to identify any potential hazards that might arise from the plant's use. Acute and sub-acute mammal toxicity test results classified A. dimidiata as non-toxic and non-irritating. Based on this toxicity evaluation, the dried leaf material and aqueous extracts of this plant are considered safe for use in preliminary field trials.

Gartzke J, Lange K, Brandt U, Bergmann J. **A new concept for risk assessment of the hazards of non-genotoxic chemicals--electronmicroscopic studies of the cell surface. Evidence for the action of lipophilic chemicals on the Ca2+ signaling system.** Sci Total Environ 1997;199(1-2):213-26.
Recently, we presented evidence for the localization of components of the cellular Ca2+ signaling pathway in microvilli. On stimulation of this pathway, microvilli undergo characteristic morphological changes which can be detected by scanning electron microscopy (SEM) of the cell surface. Here we show that both receptor-mediated (vasopressin) and unspecific stimulation of the Ca2+ signaling system by the lipophilic tumor promoters thapsigargin (TG) and phorbolmyristateacetate (PMA) are accompanied by the same type of morphological changes of the cell surface. Since stimulated cell proliferation accelerates tumor development and sustained elevation of the intracellular Ca2+ concentration is a precondition for stimulated cell proliferation, activated Ca2+ signaling is one

possible mechanism of non-genomic tumor promotion. Using isolated rat hepatocytes we show that all tested lipophilic chemicals with known tumor promoter action, caused characteristic microvillar shape changes. On the other hand, lipophilic solvents that were used as differentiating agents in cell cultures such as dimethylsulfoxide (DMSO) and dimethylformamide also, failed to change the microvillar shapes. Instead DMSO stabilized the original appearance of microvilli. The used technique provides a convenient method for the evaluation of non-genomic.

Naimark D, Krahn MD, Naglie G, Redelmeier DA, Detsky AS. **Primer on medical decision analysis: Part 5--Working with Markov processes.** Med Decis Making 1997;17(2):152-9.
Clinical decisions often have long-term implications. Analysis encounter difficulties when employing conventional decision-analytic methods to model these scenarios. This occurs because probability and utility variables often change with time and conventional decision trees do not easily capture this dynamic quality. A Markov analysis performed with current computer software programs provides a flexible and convenient means of modeling long-term scenarios. However, novices should be aware of several potential pitfalls when attempting to use these programs. When deciding how to model a given clinical problem, the analyst must weigh the simplicity and clarity of a conventional tree against the fidelity of a Markov analysis. In direct comparisons, both approaches gave the same qualitative answers.