1998 No. 1

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

TOXICOLOGY AND ENVIRONMENTAL HEALTH INFORMATION PROGRAM
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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

Diener W, Kayser D, Schlede E. **The inhalation acute toxic class method: test procedures and biometric evaluations**. Arch Toxicol 1997;71(9):537-49.
CBAC COPYRIGHT: CHEM ABS A method of inhalation acute toxic (ATC) classification is presented with the use of significantly fewer animals in comparison with the classical LC50 test. The principle of the inhalation ATC method is based on the oral ATC method, which has been adopted in 1996 as an official test guideline of the OECD and the European Union. The inhalation ATC method, like the oral ATC method, is a stepwise procedure; three animals of each sex are used simultaneously for each tested concn., and not, as in the oral ATC method, three animals of each sex sep. for each dose. The method was developed for three starting concns. and two ref. systems (based on ppm and mg/l). Depending on the LC50, slope, classification system and starting concn., on av. 50 to 80 fewer animals will be used in comparison to at least 30 animals with the classical LC50 test. The method was biometrically evaluated with the use of the probit model for dose-response relationships. At present, there are 12 different international classification systems based on LC50 values: 6 systems referring to mg/L and 6 systems based on ppm values, and exposure time varying from 1 to 4 h. The test procedures and the calcns. of the classification probabilities demonstrate that the inhalation ATC method is a reliable alternative to the classical LC50 test with the use of significantly fewer animals. Classification probabilities are presented for all classification systems currently in use, and expected nos. of exptl. and of moribund/dead animals are demonstrated for one system of each ref. system and for all three starting concns. The conclusion is justified that there is no need to validate the inhalation ATC method with the use of exptl. animals.

Kobayashi K, Ohori K, Kobayashi M, Takeuchi H. **[Choice of method for statistical analysis of quantitative data obtained from toxicological studies--toxicological data]**. Sangyo Eiseigaku Zasshi 1997;39(3):86-92. (Jpn)
We compared the usefulness of t-test and parametric and rank-sum tests in the statistical analysis of significant differences in the so-called decision tree for the quantitative data obtained from toxicity studies. The Dunnett's multiple comparison test had lower analytic power than the t-test when one of the groups showed a marked difference in variance. The Dunnett's test was less efficient with the increase in the number of groups. If one group showed a decrease in the number of animals, this test was less efficient than parametric tests, because the rank-sum tests should be chosen. The rank-sum test is required occasionally to attach the asterisks of significant difference to the mean +/- SD even in showing the same mean values. The nonparametric Dunnett's test could not be used for analysis of significant differences when the mean value for the control and treated groups showed big differences. The nonparametric Dunnett's and parametric Scheffe tests were not as efficient as the other parametric tests probably because of the vague evaluation or overlooking the effect of the test substance.

Rodford R. **Safety evaluation of preservatives.** Intl J Cosmet Sci 1997;19(6):281-90.
BIOSIS COPYRIGHT: BIOL ABS. Preservatives are added to cosmetic products to increase their shelf-life by protecting them against microbial contamination which would otherwise cause spoilage and pose

a hazard of infection to the consumer. However, preservatives are biologically active chemicals which also pose health hazards to the consumer. It is therefore important that there is both microbiological and toxicological input to the development of new product formulations which contain preservatives. Knowledge in the use of preservatives has progressed over the last 25 years from a fairly imprecise science to an understanding of the actual levels required to work effectively without causing harm to the consumer. In addition, the development of new biocidal chemicals has also progressed to the point where, although the 'perfect' preservative does not yet exist, it is possible to get close to near-perfect preservation by using the most appropriate type for the application and/or by blending to give a broader spectrum of activity. Toxicological testing has likewise gone through a rapid evolution from acute effects testing involving large numbers of animals to in vitro screening techniques which enable the safety evaluation scientist to make effective assessments of the hazards of new formulated products without the need for a large programme of animal testing for support. At the same time the safety-evaluation process has moved forward from a purely hazard-assessment approach to a risk-assessment one that evaluates the hazards against the end-use and potential misuse of a product. Using these techniques, a concentration of a preservative which will give an acceptable safety margin for the consumer in terms of both topical and systemic toxicity can be calculated. As the European Commission moves towards further reductions in animal testing we look forward to the challenge of understanding the toxic mechanisms in vivo which we are trying to model with alternative methods. The use of quantitative structure activity relationships (QSAR) will help to understand these mechanisms. Such understanding will aid in the acceptance of alternative methods into the regulatory process. This will enable us to continue to provide consumers with safe and effectively preserved personal-care products.

Sells PG, Richards AM, Laing GD, Theakston RD. **The use of hens' eggs as an alternative to the conventional in vivo rodent assay for antidotes to haemorrhagic venoms.** Toxicon 1997;35(9):1413-21.
One of the tests used routinely for the preclinical assessment of antivenom efficacy is the WHO-approved rodent intradermal skin test for assessing neutralization of venom-induced haemorrhagic activity. This is a useful test as in many viperid venoms haemorrhage is considered to be the principal lethal (pathogenic) venom effect in envenomed humans. The main problems with such an assay are, first, the necessity of using large numbers of experimental rodents (rats or mice) in order to obtain statistically significant results and, second, that the test must result in pain for the animals during the 24 hr assay period. The present study compares the rodent assay with an alternative assay using venom, in both the presence and absence of antidote, applied to a filter paper disc and placed on the highly vascularized yolk sac membrane of chickens' eggs at an early developmental stage. This avoids sensitivity to pain as reflex arcs have not yet developed, and haemorrhage or neutralization/inhibition of haemorrhage can be easily recorded. Preliminary results showed a high level of correlation between the results of the two tests when used to assess the efficacy of an antidote. It is hoped that the new assay will reduce the need for pain-sensitive experimental animals in the future.

CARCINOGENESIS

Changes to classifying carcinogens [news]. Environ Health Perspect 1997;105(10):1040-2.

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans: polychlorinated dibenzo-para-dioxins and polychlorinated dibenzofurans. Lyon, France, 4-11 February 1997. IARC Monogr Eval Carcinog Risks Hum 1997;69:1-631.

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans: silica, some silicates, coal dust and para-aramid fibrils. Lyon, 15-22 October 1996. IARC Monogr Eval Carcinog Risks Hum 1997;68:1-475.

Albert RE. **Allergic contact sensitizing chemicals as environmental carcinogens.** Environ Health Perspect 1997;105(9):940-8.
BIOSIS COPYRIGHT: BIOL ABS. Chemicals that were bioassayed by the National Toxicology Program (NTP) and that also produce allergic dermatitis (ACD) in humans were evaluated for their tumorigenic characteristics. The impetus for the study was that most contact sensitizers, i.e., those that produce ACD, and genotoxic carcinogens are chemically similar in that they are electrophilic, thereby producing adducts on macromolecules including protein and DNA. This similarity in chemical behavior suggests that many contact sensitizers might be environmental carcinogens. All of the published NTP bioassays by early 1996 that had both genotoxicity and carcinogenicity studies were included in this analysis. The NTP chemicals had been chosen for bioassay without regard to their ability to produce ACD. Of the 209 chemicals that were bioassayed, there were 36 (17%) that were known to be human contact sensitizers; about half of these were positive on tumor bioassays. The contact sensitizers differed from the NTP sample as a whole by having a proportionately larger number of nongenotoxic chemicals by the Ames Salmonella assay, presumably because more of them were selected on the basis of widespread usage rather than structural resemblance to known carcinogens. Compared to the nongenotoxic chemicals, the genotoxics were stronger carcinogens in that they had a higher incidence of positive tumor bioassays, with twice the number of organs in which tumors were induced. The nongenotoxic chemicals had a preference for tumor induction in parenchymal tissues in contrast to epithelial tissues. The contact sensitizers showed essentially the same characteristics as the whole NTP sample when stratified according to genotoxicity. Judging by the chemicals that were chosen primarily for their widespread use rather than for their structural resemblance to carcinogens, the addition of a test for contact sensitization to the Ames test as a screening tool would increase the tumorigenic detection efficiency by about 40% because of the nongenotoxic tumorigens. A ballpark estimate suggests that there could be several thousand contact sensitizers for humans in commercial use that are rodent tumorigens.

Aruin LI. **[Helicobacter pylori infection is carcinogenic for humans]**. Arkh Patol 1997;59(3):74-8. (Rus)
International Agency for Research on Cancer recognized as sufficient the evidence of Helicobacter pylori (HP) infection carcinogenicity and placed it into the 1st group of carcinogens. The results of the studies performed during last years are discussed. They showed the involvement of HP in the development of stomach cancer. HP enhances mitogenesis of epithelium with translocation of immature cells on the surface of stomach mucous membrane and thus forms the targets for mutagenic and carcinogenic influences. HP takes part in the formation of mutagens, primarily of free oxygen radicals. Achlorohydria is the outcome of HP-gastritis and results in the bacterial growth in the stomach which

participate in the formation of N-nitrosocompounds-well known carcinogens. Antitumor stomach defense is weakened by the decrease in the stomach of ascorbic acid, carotin and tocopherol resulting from HP-infection.

Baijal PK, Fitzpatrick DW, Bird RP. **Phenobarbital and 3-methylcholanthrene treatment alters phase I and II enzymes and the sensitivity of the rat colon to the carcinogenic activity of azoxymethane.** Food Chem Toxicol 1997;35(8):789-98.
It has been hypothesized that cancer risk may be influenced by phase I and II drug-metabolizing enzyme systems. This study attempted to determine the relationship between colon phase I and II enzyme activity and the subsequent induction of aberrant crypt foci (ACF), preneoplastic lesions by azoxymethane (AOM), a colon-specific carcinogen. Phenobarbital (PB) and 3-methylcholanthrene (MC) treatment (prototype hepatic inducers of phase I and II enzymes) provided the framework to study the induction of phase I and II enzymes in the rat colonic mucosa. Following induction for five consecutive days, the animals were given a single injection of AOM. Phase I and II enzymes were determined fluorometrically and spectrophotometrically and ACF were identified microscopically. Phase I and II xenobiotic metabolizing enzymes were induced in the rat colonic mucosa by prototype hepatic inducers. A lower number of ACF and crypt multiplicity was observed in animals induced with MC than in those in the non-induced and PB groups. Altered levels of phase I and II enzymes in the colon during preinitiation stages were associated with modulation in the growth of ACF, putative preneoplastic lesions.

Becker SA, Lee TH, Butel JS, Slagle BL. **Hepatitis B virus X protein interferes with cellular DNA repair.** J Virol 1998;72(1):266-72.
The hepatitis B virus X protein (HBx) is a broadly acting transactivator implicated in the development of liver cancer. Recently, HBx has been reported to interact with several different cellular proteins, including our report of its binding to XAP-1, the human homolog of the simian repair protein UVDDB. In the present study, several HBx mutants were used to localize the minimal domain of HBx required for binding to XAP-1/UVDDB to amino acids 55 to 101. The normal function of XAP-1/UVDDB is thought to involve binding to damaged DNA, the first step in nucleotide excision repair (NER); therefore, we hypothesized that this interaction may affect the cell's capacity to correct lesions in the genome. When tested in two independent assays that measure NER (unscheduled DNA synthesis and host cell reactivation), the expression of HBx significantly inhibited the ability of cells to repair damaged DNA. Under the assay conditions, HBx was expressed at a level similar to that previously observed during natural viral infection and was able to transactivate several target reporter genes. These results are consistent with a model in which HBx acts as a cofactor in hepatocarcinogenesis by preventing the cell from efficiently repairing damaged DNA, thus leading to an accumulation of DNA mutations and, eventually, cancer. An adverse effect on cellular DNA repair processes suggests a new mechanism by which a tumor-associated virus might contribute to carcinogenesis.

Bogdanffy MS, Mathison BH, Kuykendall JR, Harman AE. **Critical factors in assessing risk from exposure to nasal carcinogens.** Mutat Res 1997;380(1-2):125-41.
Anatomical, physiological, biochemical and molecular factors that contribute to chemical-induced nasal carcinogenesis are either largely divergent between test species and humans, or we know very little of

them. These factors, let alone the uncertainty associated with our knowledge gap, present a risk assessor with the formidable task of making judgments about risks to human health from exposure to chemicals that have been identified in rodent studies to be nasal carcinogens. This paper summarizes some of the critical attributes of the hazard identification and dose-response aspects of risk assessments for nasal carcinogens that must be accounted for by risk assessors in order to make informed decisions. Data on two example compounds, dimethyl sulfate and hexamethylphosphoramide, are discussed to illustrate the diversity of information that can be used to develop informed hypotheses about mode of action and decisions on appropriate dosimeters for interspecies extrapolation. Default approaches to interspecies dosimetry extrapolation are described briefly and are followed by a discussion of a generalized physiologically based pharmacokinetic model that, unlike default approaches, is flexible and capable of incorporating many of the critical species-specific factors. Recent advancements in interspecies nasal dosimetry modeling are remarkable. However, it is concluded that without the development of research programs aimed at understanding carcinogenic susceptibility factors in human and rodent nasal tissues, development of plausible modes of action will lag behind the advancements made in dosimetry modeling.

Bottinger EP, Jakubczak JL, Haines DC, Bagnall K, Wakefield LM. **Transgenic mice overexpressing a dominant-negative mutant type II transforming growth factor beta receptor show enhanced tumorigenesis in the mammary gland and lung in response to the carcinogen 7,12-dimethylbenz-[a]-anthracene.** Cancer Res 1997;57(24):5564-70.
To test the hypothesis that the transforming growth factor-beta (TGF-beta) system has tumor suppressor activity in the mammary gland, we have generated transgenic mice overexpressing a dominant-negative mutant form of the type II TGF-beta receptor, under the control of the mouse mammary tumor virus-long terminal repeat. High-level expression of the transgene was observed in the mammary and salivary glands, with lower expression in the lung, spleen, and testis. Older nulliparous transgenic mice (9-17 months) showed a marked increase in the incidence and degree of lobulo-alveolar side-branching in the mammary glands when compared to wild-type littermates (24.8% of glands examined histologically versus 14.4%; P = 0.004), suggesting a role for endogenous TGF-betas in regulating development or maintenance of mammary alveoli. Spontaneous tumorigenesis was unchanged in the transgenic mice. However, following initiation with the carcinogen 7,12-dimethylbenz[a]anthracene, the transgenic group showed a significant increase in the incidence and multiplicity of mammary tumors when compared with wild-type littermates (40% incidence in transgenic mice versus 22% for wild-type, with 4 of 25 transgenics developing multiple mammary tumors versus 0 of 27 wild-type; P = 0.03). An early increase in the incidence of lung tumors was also observed in transgenic mice, but no difference between genotype groups was seen in the incidence of tumors in tissues in which the transgene is not expressed. The data show that the endogenous TGF-beta system has tumor suppressor activity in the mammary gland and lung.

Carmichael NG, Enzmann H, Pate I, Waechter F. **The significance of mouse liver tumor formation for carcinogenic risk assessment: results and conclusions from a survey of ten years of testing by the agrochemical industry.** Environ Health Perspect 1997;105(11):1196-203.
A survey was performed on the results of 138 carcinogenicity studies conducted in various mouse strains by the agrochemical industry over the period 1983-1993. Data for liver tumor incidence, liver weight,

and histopathology were collected along with data on genotoxicity. Studies were judged positive or negative for liver tumor formation on the basis of apparent dose response, malignancy, and difference from historical control values using a weight of evidence approach. Thirty-seven studies were judged to be positive for liver tumorigenicity in one or both sexes. There was no evidence showing an influence of the mouse strain and the duration of the study on the proportion of positive studies. Although 8 of the chemicals tested in the 138 studies were positive in the Ames test, only one of these was judged positive for carcinogenicity. Only 6 of the 37 positive chemicals had any other reported positive genotoxicity findings. A clear relationship between hepatomegaly at 1 year after exposure and a positive tumorigenic outcome at 18 months or 2 years after exposure was demonstrated. Whereas the average relative liver weight of top dose animals was 110% of control in negative studies, it was 150% in positive studies. Likewise, very few negative studies demonstrated significant pathological findings after 1 year, whereas the majority of positive studies had significant liver pathology. The implications of these findings for extrapolation to humans are discussed.

Casale GP, Higginbotham S, Johansson SL, Rogan EG, Cavalieri EL. **Inflammatory response of mouse skin exposed to the very potent carcinogen dibenzo(a,l)pyrene: a model for tumor promotion**. Fundam Appl Toxicol 1997;36(1):71-8.
The time course of the dermatotoxicity of dibenzo(a,l)pyrene (191300) (DBalP) was studied in mice. Six week old female SENCAR-mice were administered up to 200 nanomoles (nmol) DBalP, dibenzo(a,l) pyrene-11,12-dihydrodiol (DBalp11,12diol), dibenzo(a,l)pyrene-8,9-dihydrodiol (DBalp89diol), or the syn or anti isomers of dibenzo(a,l)pyrene-11,12-dihydrodiol-13,14-epoxide (dBalPDE) topically. The treated skin areas were evaluated for inflammation by examining and scoring them for erythema periodically up to 35 days after dosing. Selected mice were killed at various times after dosing and the treated skin areas were excised and examined for histopathological changes. DBalP and DBalP11,12diol induced an erythema that was first observed 5 to 6 days after application and which peaked in intensity at 7 to 10 days. This early time course did not depend on the dose of either DBalP or DBalP11,12diol. The intensity and duration of the erythemic response was dose dependent. The erythema subsided 14 days after DBalP and 21 days after DBalp11,12diol. DBalP89diol and the syn and anti isomers of dBalPDE did not induce erythema. The time course of the erythemic changes was paralleled histologically by induction of epidermal hyperplasia and infiltration of the dermis by mononuclear leukocytes. Because these changes resembled a contact hypersensitivity reaction, additional SENCAR-mice were treated topically with 0, 200, or 400nmol DBalP or 7,12-dimethylbenz(a)anthracene (57976) (DMBA) then challenged 5 days later with 80nmol DBalP or DMBA applied to the ear pinna. Induction of contact hypersensitivity was assessed by measuring the amount of swelling of the ear. The DBalP challenge doses induced significant delayed hypersensitivity 3 to 4, and 5 to 7 days after challenge of DBalP initiated mice with DBalP. DMBA induced no response. The authors conclude that the skin inflammation induced by DBalP contributes significantly to its carcinogenic activity by way of tumor promotion mediated by dBalPDE.

Clayson DB. **Carcinogen risk assessment: a necessary dilemma?** Cancer Lett 1997;117(2):209-15.
Carcinogen risk assessment is the process by which an attempt is made to estimate human risk due to carcinogens, from the results of animal studies. It is based upon a number of prudent default assumptions, that is, assumptions that cannot be proved scientifically because either the basic concept is

philosophical in nature or because the amount of scientific evidence required is too costly to obtain even on a world-wide basis. Recently, scientific effort has shown that more and more examples have been described suggesting these examples do not behave in the way indicated by the default assumptions. Since carcinogen risk assessment processes were initiated, it has been demonstrated that cancer may arise by four or more different mechanisms. It is the purpose of this paper to enquire whether consideration of these basically different mechanisms may facilitate carcinogen risk assessment.

Cox LA Jr. **Reassessing benzene risks using internal doses and Monte-Carlo uncertainty analysis**. Environ Health Perspect 1996;104(Suppl 6):1413-29.
The method by which inferences concerning low dose human cancer risks traditionally have been drawn from animal bioassay data was reviewed. The author then suggests that a different approach is needed for benzene (71432) since benzene biology is not consistent with the assumptions found in the usual approach. Use of Monte Carlo uncertainty analysis, use of two species specific physiologically based pharmacokinetics models, and use of a modeling framework for incorporating additional biological knowledge were discussed. The traditional statistical risk assessment of benzene was discussed including the selection of a dose metric, parameter estimation via maximum likelihood, uncertainty analysis, and extrapolating risk from oral gavage data for mice to inhalation hazards for humans. Methods for improving benzene risk assessment included causal decomposition of the cancer risk process, maximum entropy Bayesian Monte Carlo uncertainty analysis, and biologically based computer simulation models. The author concludes that there is no evidence of a positive relation between benzene exposure and tumor probability at benzene concentrations lower than 1 part per million; that different analytic approaches suggest that the curve relating benzene concentration to AML risk at sufficient low constant concentrations of benzene approach a zero or negative slope as concentration falls below 10 parts per million, and empirical data and biologically based risk models suggest that higher concentrations of benzene may cause disproportionately large hepatotoxic responses for the same total administered dose.

Duthie SJ, Mcmillan P. **Uracil misincorporation in human DNA detected using single cell gel electrophoresis**. Carcinogenesis 1997;18(9):1709-14.
CBAC COPYRIGHT: CHEM ABS Poor folate status may be important in the etiol. of several epithelial cell malignancies including cancer of the uterine cervix. Folic acid is essential in the synthesis of purine nucleotides and the pyrimidine nucleoside thymidine and it is probable that imbalances in these DNA precursors neg. effect DNA stability and may ultimately lead to malignant transformation. The development of a modified comet assay using the bacterial DNA repair enzyme uracil DNA glycosylase, to detect misincorporated uracil in human DNA is reported here. The effect of perturbing folic acid and deoxyuridine levels on uracil misincorporation in normal human lymphocytes and cultured human tumor cells was investigated using this assay. HeLa cells and peripheral human lymphocytes incubated as agarose-embedded nucleoids, with 1 unit of uracil DNA glycosylase per mug of DNA, contained low levels of uracil in their DNA. Both HeLa cells and stimulated human lymphocytes cultured in folate-deficient medium were growth arrested. Incubating human lymphocytes in folate-deficient medium significantly increased the level of uracil detected compared with control cells. HeLa cells showed an increase in non-specific DNA damage (strand breaks). Deoxyuridine (100 muM) significantly increased the level of uracil detected in the DNA of both folate-deficient and control HeLa cells. It appears that

this modified comet assay specifically detects misincorporated uracil in single human cells. It should, therefore, prove valuable in detg. the role of folic acid status in DNA instability and cancer.

Flamm WG, Hughes D. **Does the term carcinogen send the wrong message?** Cancer Lett 1997;117 (2):189-94.
The term carcinogen has been used by scientists and health regulatory officials for decades. During the last 20 years there have been attempts to redefine the term to make it more rigorous. But, as predicted two decades ago by a benchmark-setting subcommittee of the National Cancer Advisory Board, advances in scientific understanding have brought about dramatic changes in the way we are able to view the term carcinogen. These changes, their scientific bases and their effect on defining the term carcinogen are described. An alternative to the use of the term carcinogen is suggested by the recently proposed US Environmental Agency's guidelines for cancer risk assessment which appear to be in accord with current scientific understanding and the importance of considering the factors affecting the term carcinogen. The guidelines set forth four questions, the answers to which could, in our judgment, replace the need to define or use the term carcinogen which, in light of new scientific knowledge, has become more misleading than useful.

Flammang TJ, Tungeln LS, Kadlubar FF, Fu PP. **Neonatal mouse assay for tumorigenicity: alternative to the chronic rodent bioassay.** Regul Toxicol Pharmacol 1997;26(2):230-40.
The chronic rodent bioassay for tumors has been utilized systematically for 25 years to identify chemicals with carcinogenic potential in man. In general, those chemicals exhibiting tumorigenicity at multiple sites in both mice and rats have been regarded as possessing strong carcinogenic potential in humans. In comparison, the value of data collected for those test chemicals exhibiting more sporadic tumorigenicity results (e.g., single species/single sex or dose-independent) has been questioned. As knowledge of the carcinogenic process has increased, several alternative test systems, usually faster and less expensive than the 2-year bioassay, have been suggested for identification of the strongly acting, transspecies carcinogens. The International Conference on Harmonization for Technical Requirements for the Registration of Pharmaceuticals for Human Use has proposed an international standard that allows for the use of one long-term rodent carcinogenicity study, plus one supplementary study to identify potential human pharmaceutical carcinogens. The neonatal mouse assay for tumorigenicity has been used since 1959; however, relative to other alternate tests, little has been written about this system. It is clear that this assay system successfully identifies transspecies carcinogens from numerous chemical classes, thus recommending itself as a strong candidate for a supplementary study to identify potential human carcinogens. In contrast, there are decidedly less data available from this assay in response to pharmaceuticals shown to exhibit weak and/or conflicting results in the 2-year bioassay, knowledge invaluable to the regulatory process. This paper reviews the historical development and our experience with the neonatal mouse assay and includes suggestions for a standardized protocol and strategies to document its response to weak and/or nongenotoxic carcinogens. Copyright 1997 Academic Press.

Fuscoe JC, Setzer RW, Collard DD, Moore MM. **Quantification of t(14;18) in the lymphocytes of healthy adult humans as a possible biomarker for environmental exposures to carcinogens**. Carcinogenesis 1996;17(5):1013-20.

A quantitative nested polymerase chain reaction (PCR) technique was developed to determine the peripheral blood lymphocyte frequency of a t(14;18) chromosomal translocation in healthy adults as a biological marker for environmental exposure to carcinogens. Blood samples were collected from 34 healthy young adult donors; lymphocytes were isolated and DNA samples were prepared. The t(14;18) junctions in the peripheral blood lymphocyte DNA were amplified using a nested PCR method, and DNA sequence analysis was conducted on the PCR products generated with t(14;18) translocation specific primers to determine the DNA sequence of the junction region, which included the bcl-2 breakpoint, location of insertion in the immunoglobulin heavy chain locus, and the N-region. Statistical analyses were conducted to determine the frequency of translocations per cell and whether the frequencies of cells with translocations were different between men and women. Peripheral blood cells containing the t(14;18) translocation were detected in 88% of the subjects, and the frequency of these cells ranged from less than 0.8 to 96x10(-7). Multiple isolates of the t(14;18) translocation event were recovered in several individuals, which suggested that the cell with the original translocation had undergone clonal expansion. Multiple independent translocations were also found in one subject. The authors conclude that this assay may be used as a biomarker for exposure to environmental carcinogens, since the t(14;18) translocation seems to be one of the steps involved in the progression of a normal cell to a cancer cell.

Golden RJ, Holm SE, Robinson DE, Julkunen PH, Reese EA. **Chloroform mode of action: implications for cancer risk assessment.** Regul Toxicol Pharmacol 1997;26(2):142-55.
Risk assessment methodology, particularly pertaining to potential human carcinogenic risks from exposures to environmental chemicals, is undergoing intense scrutiny from scientists, regulators, and legislators. The current practice of estimating human cancer risk is based almost exclusively on extrapolating the results of chronic, high-dose studies in rodents to estimate potential risk in humans. However, many scientists are questioning whether the logic used in this current risk assessment methodology is the best way to safeguard public health. A major tool of human cancer risk assessment is the linearized multistage (LMS) model. The LMS model has been identified as an aspect of risk assessment that could be improved. One way to facilitate this improvement is by developing a way to incorporate a carefully derived, more biologically relevant mechanism of action data on carcinogenesis. Recent data on chloroform indicate that the dose-response relationship for chloroform-induced tumors in rats and mice is nonlinear, based upon events secondary to cell necrosis and subsequent regeneration as the likely mode of action for the carcinogenic effects of chloroform. In light of these data, there is a sound scientific basis for removing some of the uncertainty that accompanies current cancer risk assessments of chloroform. The following points summarize the critical data: (1) a substantial body of data demonstrates a lack of direct in vivo or in vitro genotoxicity of chloroform; (2) chloroform induces liver and kidney tumors in long-term rodent cancer bioassays only at doses that induce frank toxicity at these target sites; (3) the chloroform doses required to produce tumors in susceptible species exceed the MTD, often by a considerable margin; (4) cytotoxicity and compensatory cell proliferation are associated with the chloroform doses required to induce liver or kidney tumors in susceptible rodent species; (5) there are no instances of chloroform-induced tumors that are not preceded by this pattern of dose-dependent toxic responses; (6) it is biologically plausible that cytolethality leads to chronically stimulated cell proliferation and related events such as inflammation and growth stimulation which act to initiate and promote the carcinogenic process; and (7) the consistently linked cellular events of

cytolethality and subsequent cell proliferation, for which doses of no adverse effect have been clearly shown to exist, are one of the biological prerequisites for chloroform-induced tumors in animals. Based on these data, it is inappropriate to extrapolate cancer risk from high doses that produce necrosis and regenerative cell proliferation to low doses that do not with a model that presumes genotoxicity and a linear dose-response relationship. The weight of the scientific evidence concerning chloroform-induced tumors in rodents is consistent with and supports a cancer risk assessment methodology based on mode of action as the basis for establishing regulatory standards for this compound. Copyright 1997 Academic Press.

Haseman JK, Hailey JR. **An update of the National Toxicology Program database on nasal carcinogens.** Mutat Res 1997;380(1-2):3-11.
Nearly 500 long-term rodent carcinogenicity studies carried out by the National Cancer Institute and the National Toxicology Program were examined, and 12 chemicals were identified that produced nasal tumors: allyl glycidol ether, p-cresidine, 1,2-dibromo-3-chloropropane, 1,2-dibromoethane, 2,3-dibromo-1-propanol, dimethylvinyl chloride, 1,4-dioxane, 1,2-epoxybutane, iodinated glycerol, procarbazine, propylene oxide, and 2,6-xylidine. All 12 of these chemicals produced nasal tumors in rats, and 5 also produced nasal tumors in mice. Most of the nasal carcinogens (1) produced tumor increases in both sexes, (2) produced tumors at other sites as well, (3) had significantly reduced survival at doses that were carcinogenic, and (4) were genotoxic. Only 5 of the 12 nasal carcinogens were administered by inhalation. A variety of different types of nasal cavity tumors were produced, and specific tumor rates are given for those chemicals causing multiple tumor types. Increased incidences of nasal neoplasms were often accompanied by suppurative/acute inflammation, epithelial/focal hyperplasia and squamous metaplasia. However, high incidences of these nonneoplastic nasal lesions were also frequently seen in inhalation studies showing no evidence of nasal carcinogenicity, suggesting that in general nasal carcinogenesis is not associated with the magnitude of chronic toxicity observed at this site.

Hong JY, Yang CS. **Genetic polymorphism of cytochrome P450 as a biomarker of susceptibility to environmental toxicity.** Environ Health Perspect 1997;105(Suppl 4):759-62.
Cytochrome P450 (CYP) enzymes are responsible for the metabolism of numerous xenobiotics and endogenous compounds, including the metabolic activation of most environmental toxic chemicals and carcinogens. Both metabolic and genetic polymorphisms have been identified for human CYP enzymes. The association of CYP genetic polymorphism and human cancer risk, and susceptibility to environmental hazards, have received increasing attention. This article briefly reviews the approaches and methods currently used in CYP genetic polymorphism studies. In addition, the current status and perspectives of using CYP genetic polymorphism as a biomarker of individual susceptibility to cancer and environmental toxicity are discussed.

Humfrey CD, Levy LS, Faux SP. **Potential carcinogenicity of foundry fumes: a comparative in vivo-in vitro study**. Food Chem Toxicol 1996;34(11-12):1103-11.
In-vivo and in-vitro studies were conducted to evaluate the potential carcinogenicity of fumes from three of the most mutagenic foundry sand binder systems. A 2 year chronic in-vivo study in rats used an intrabronchial pellet implantation technique to study pathological changes in the bronchial epithelium. The second part of the study included the development and use of a number of short term in-vitro assays,

to assess cytotoxicity and the potential genotoxicity of fumes in cultured tracheal epithelial cells. The toxicity and genotoxicity of the fumes were tested concurrently in a number of in-vitro assays including those identifying mutagenicity, unscheduled DNA synthesis, free radical DNA damage and micronucleus induction. The authors conclude that the in-vitro methods used in the present study to assess genotoxicity of a complex mixture have endpoints that can be used as biomarkers for potential carcinogenicity. Although in-vitro tests cannot mimic all the events occurring in a whole animal and therefore can never totally replace in-vivo testing, their continued improvement will make them more suitable for risk assessment and may reduce the numbers of animals needed for experimentation.

Indulski JA, Lutz W. **Molecular epidemiology: cancer risk assessment using biomarkers for detecting early health effects in individuals exposed to occupational and environmental carcinogens.** Rev Environ Health 1997;12(3):179-90.
Detecting changes that precede the overt symptoms of cancer and identifying measurable indices of such changes in persons exposed to occupational and environmental carcinogens constitutes one of the primary objectives of molecular epidemiology research. Biomarkers represent a valuable research tool that makes it possible to attain that objective. Suitably selected biomarker sets may provide information on the extent of exposure to carcinogenic agents (internal dose, biologically effective dose), detect early changes caused by those agents in the exposed organism, and identify individuals with a particularly high risk of cancer development. The tremendous progress in research on the mechanisms of cancer initiation and promotion has enabled the assessment of cancer risk in healthy individuals by examining specific results from determinations of suitably selected biomarkers. The finding that gene defects (gene mutations and changes of their expression) constitute the background of carcinogenesis has resulted in molecular biology becoming focused on detecting defective genes or proteins synthesized under control of the defective genes.

Kajiwara Y, Ajimi S, Hosokawa A, Maekawa K. **Improvement of carcinogen detection in the BALB/3T3 cell transformation assay by using a rich basal medium supplemented with low concentration of serum and some growth factors.** Mutat Res 1997;393(1-2):81-90.
To improve the detection sensitivity and reproducibility of the transformation assay using BALB/3T3 cells, we scrutinized a new assay method in which the cells were replated in a medium containing a low concentration of serum after carcinogen treatment. Dulbecco's modified Eagle's medium plus Ham F12 (DME.F12) supplemented with a mixture of insulin, transferrin, ethanolamine and sodium selenite (ITES) and a low concentration of fetal calf serum (FCS) caused transformation at a high frequency with a high reproducibility. The transformation frequency in the culture treated with N-methyl-N'-nitro-N-nitrosoguanidine was the highest in DME.F12 medium containing ITES and 2% FCS, and it decreased at either a lower or higher FCS concentration. Moreover, the transformation frequency was not markedly influenced by the difference in lot of FCS, probably due to reduction in FCS concentration. According to the present method, the transformation frequency was 2-times higher and transformed foci appeared much earlier than by the method using the original medium. Next, we examined some geno- and non-genotoxic carcinogens, and some genotoxic non-carcinogens to confirm the availability of this method for predicting potential carcinogens. This method could precisely identify not only genotoxic carcinogens but also non-genotoxic carcinogens and genotoxic non-carcinogens. In conclusion, these findings suggest that this method is useful for detection of chemical carcinogens because it provides

high sensitivity, high reproducibility and accurate predictivity, without the requirement of 12-O-tetradecanoylphorbol 13-acetate as a promoter, making it harmless to examiner.

Kolar C, Lawson T. **Mutagenicity of carcinogenic nitrosamines when activated by hamster and human pancreatic duct epithelial cells.** Cancer Lett 1997;117(2):149-54.
We have measured the ability of pancreatic duct epithelial cells (DEC) from Syrian hamsters and humans and CK cells, immortalized hamster DEC, to metabolize chemical carcinogens to species that were mutagenic in S. typhimurium TA98 and in V79 cells. The chemicals were N-nitrosobis(2-oxopropyl)amine (BOP), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). The ability of ethanol (EtOH) to modify the metabolizing efficiency was also measured. When an S9 preparation from EtOH-treated CK cells was used to metabolize NNK the number of revertants was 271 +/- 73 compared with 17 +/- 2 when the S9 from control CK cells was used. When hamster DEC were used there was no increase in the mutation frequency for BOP in V79 cells (64 +/- 20 mutants/10(6) survivors per mumol) when EtOH-DEC were used. However, the mutation frequencies of NNK and PhIP rose when the EtOH-treated DEC were used from 62 +/- 31 to 198 +/- 28 mutants/10(6) survivors per mumol for NNK and from 94 +/- 25 to 166 +/- 25 mutants/10(6) survivors per mumol for PhIP. A similar result was obtained when human DEC were used, i.e. no change in BOP mutagenicity and a slight increase in PhIP mutagenicity, from 34 +/- 14 to 65 +/- 12 mutants/10(6) survivors per mumol. There were large increases in the mutagenicity of NNK with each of the three samples of human DEC that were used, from 75 +/- 0 to 213 +/- 38, 75 +/- 13 to 175 +/- 25 and 38 +/- 13 to 285 +/- 25 mutants/10(6) survivors per mumol. The EtOH treatment regimen that was used more closely mimicked chronic exposure at low concentrations in vivo. These data show that hamster DEC are capable of metabolizing NNK, which is carcinogenic in these cells in vivo. Furthermore, human DEC metabolized NNK as efficiently as hamster DEC.

Marionnet C, Armier J, Sarasin A, Stary A. **Cyclobutane pyrimidine dimers are the main mutagenic DNA photoproducts in DNA repair-deficient trichothiodystrophy cells.** Cancer Res 1998;58(1):102-8.
We have used the replicating shuttle vector pR2 to determine the role of ultraviolet C (UVC)-induced cyclobutane pyrimidine dimers (CPDs) and nondimer photoproducts in mutagenesis in human trichothiodystrophy (TTD) cells and in their repair-proficient counterparts obtained after complementation with the wild-type XPD/ERCC2 repair gene (TTD + ERCC2 cells). Before transfection in human cells, the UVC-irradiated vector DNA was treated with Anacystis nidulans photolyase [photoreactivation (PR) procedure] that selectively removed CPDs, leaving nondimer photoproducts intact. The mutant frequency of the UV-irradiated pR2 plasmid treated by PR was similar after replication in TTD or in TTD + ERCC2 cells. This result indicates that TTD cells were able to repair nondimer photoproducts as efficiently as TTD cells complemented with the wild-type repair gene and that in TTD cells, CPDs were the major photoproducts generating an increased mutant frequency after UVC irradiation. Sequence analysis of > 300 mutant plasmids indicated that PR of the DNA increased the relative level of tandem mutations and decreased the relative level of multiple mutations in TTD cells. In both cell lines, we observed that CPDs mostly led to GC-AT transitions; whereas only nondimer photoproducts were responsible for the induction of GC-TA transversions in TTD and TTD + ERCC2 cells.

Mauderly JL. **Relevance of particle-induced rat lung tumors for assessing lung carcinogenic hazard and human lung cancer risk.** Environ Health Perspect 1997;105(Suppl 5):1337-46.
BIOSIS COPYRIGHT: BIOL ABS. Rats and other rodents are exposed by inhalation to identify agents that might present hazards for lung cancer in humans exposed by inhalation. In some cases, the results are used in attempts to develop quantitative estimates of human lung cancer risk. This report reviews evidence for the usefulness of the rat for evaluation of lung cancer hazards from inhaled particles. With the exception of nickel sulfate, particulate agents thought to be human lung carcinogens cause lung tumors in rats exposed by inhalation. The rat is more sensitive to carcinogenesis from nonfibrous particles than mice or Syrian hamsters, which have both produced false negatives. However, rats differ from mice and nonhuman primates in both the pattern of particle retention in the lung and alveolar epithelial hyperplastic responses to chronic particle exposure. Present evidence warrants caution in extrapolation from the lung tumor response of rats to inhaled particles to human lung cancer hazard, and there is considerable uncertainty in estimating unit risks for humans from rat data. It seems appropriate to continue using rats in inhalation carcinogenesis assays of inhaled particles, but the upper limit of exposure concentrations must be set carefully to avoid false-positive results. A positive finding in both rats and mice would give greater confidence that an agent presents a carcinogenic hazard to man, and both rats and mice should be used if the agent is a gas or vapor. There is little justification for including Syrian hamsters in assays of the intrapulmonary carcinogenicity of inhaled agents.

McDougal A, Wilson C, Safe S. **Induction of estradiol 2-hydroxylase activity in MCF-7 human breast cancer cells by pesticides and carcinogens.** Environ Toxicol Pharmacol 1997;3(3):195-9.
BIOSIS COPYRIGHT: BIOL ABS. The induction of 17beta-estradiol (E2) 2-hydroxylase activity was investigated in MCF-7 human breast cancer cells using 2-(3H)E2 as the substrate in a radiometric assay. Treatment of MCF-7 cells with 10 muM indole-3-carbinol (I3C) for 48 h caused a 3.5-fold induction of E2 2-hydroxylase activity, whereas, 13C at concentrations as high as 100 muM did not induce CYP1A1 mRNA levels or immunoreactive protein. Thus, the induction of E2 2-hydroxylase activity using the radiometric assay was not dependent on induction of CYP1A1. E2 2-hydroxylase activity was also increased by I3C within 2 h after treatment suggesting in situ interactions with the cellular cytochrome P450 system. The time-dependent effects of various chlorinated pesticides, antiestrogens and mammary carcinogens on E2 2-hydroxylase activity were also investigated. p,p'-DDE, atrazine and the mammary carcinogen 7,12-dimethylbenz(a)anthracene (DMBA) significantly decreased E2 2-hydroxylase activity after 2 h; whereas, only the latter two compounds decreased activity after 48 h. Both 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and the mammary carcinogen benzo(a)pyrene (BaP) induced E2 2-hydroxylase in MCF-7 cells after incubation for 48 h and this was also paralleled by induction of CYP1A1 protein. The antiestrogens ICI 164384 and ICI 182780 decreased E2 2-hydroxylase activity in MCF-7 cells after incubation for 48 h, whereas tamoxifen and 4-hydroxytamoxifen were inactive. The results indicate that chemical-induced modulation of E2 2-hydroxylase activity in MCF-7 cells is complex and does not predict their activity as mammary carcinogens.

Melendez-Colon VJ, Smith CA, Seidel A, Luch A, Platt KL, Baird WM. **Formation of stable adducts and absence of depurinating DNA adducts in cells and DNA treated with the potent carcinogen dibenzo[a,l]pyrene or its diol epoxides.** Proc Natl Acad Sci U S A 1997;94(25):13542-7.

Polycyclic aromatic hydrocarbons (PAH) are widespread environmental contaminants, and some are potent carcinogens in rodents. Carcinogenic PAH are activated in cells to metabolites that react with DNA to form stable covalent DNA adducts. It has been proposed [Cavalieri, E. L. & Roger, E. G. (1995) Xenobiotica 25, 677-688] that unstable DNA adducts are also formed and that apurinic sites in the DNA resulting from unstable PAH adducts play a key role in the initiation of cancer. The potent carcinogen dibenzo[a,l]pyrene (DB[a, l]P) is activated in cells to (+)-syn- and (-)-anti-DB[a,l]P-11, 12-diol-13,14-epoxide (DB[a,l]PDE), which have been shown to form stable adducts with DNA. To evaluate the importance of unstable PAH adducts, we compared stable adduct formation to apurinic site formation. Stable DB[a,l]PDE adducts were determined by 33P-postlabeling and HPLC. To measure apurinic sites they were converted to strand breaks, and these were monitored by examining the integrity of a particular restriction fragment of the dihydrofolate reductase gene. The method easily detected apurinic sites resulting from methylation by treatment of cells or DNA with dimethyl sulfate or from reaction of DNA with DB[a,l]P in the presence of horseradish peroxidase. We estimate the method could detect 0.1 apurinic site in the 14-kb fragment examined. However, apurinic sites were below our limit of detection in DNA treated directly with (+)-syn- or (-)-anti-DB[a,l]PDE or in DNA from Chinese hamster ovary B11 cells so treated, although in these samples the frequency of stable adducts ranged from 3 to 10 per 14 kb. We also treated the human mammary carcinoma cell line MCF-7 with DB[a,l]P and again could not detect significant amounts of unstable adducts. These results indicate that the proportion of stable adducts formed by DB[a,l]P activated in cells and its diol epoxides is greater than 99% and suggest a predominant role for stable DNA adducts in the carcinogenic activity of DB[a,l]P.

Monticello TM, Morgan KT. **Chemically-induced nasal carcinogenesis and epithelial cell proliferation: a brief review.** Mutat Res 1997;380(1-2):33-41.
BIOSIS COPYRIGHT: BIOL ABS. An increased rate of cell proliferation has long been recognized as an important factor in both human and experimental carcinogenesis, and may be a major risk factor for cancer development in a number of tissues. Limited information exists, however, regarding the relevance of increased cell proliferation and nasal cancer. Examples of toxicological studies utilizing nasal cell proliferation data as an important endpoint are briefly reviewed. Data for one of the most extensively studied chemicals, the weakly genotoxic carcinogen formaldehyde, support the contention that the concentration-response relationship for tumor incidence is a function of formaldehyde-induced target cell proliferation, in addition to other factors including target cell population size. The increasing importance of utilizing cell proliferation data in determining dose-response relationships and in biologically-based risk assessment models is discussed.

Murata JI, Tada M, Iggo RD, Sawamura Y, Shinohe Y, Abe H. **Nitric oxide as a carcinogen: analysis by yeast functional assay of inactivating p53 mutations induced by nitric oxide.** Mutat Res 1997;379 (2):211-8.
BIOSIS COPYRIGHT: BIOL ABS. We have used a yeast p53 functional assay to study induction of mutations in the p53 tumor suppressor gene by nitric oxide oxide and cytosine methylation. The yeast assay identifies only biologically important p53 mutations. p53 cDNA was with the nitric oxide donor sydnonimine, PCR-amplified and transfected into yeast. Sydnonimine produced a significant, dose-dependent increase in C:G - A:T transversions. Many important p53 mutational hotspots are postulated to arise by deamination of methylCpG in tumors. We therefore examined nitric oxide induction of

mutations in p53 cDNA methylated by PCR-mediated substitution of 5-methylcytosine for cytosine or by treatment with the SssI CpG methylase. Both methylation procedures increased the baseline mutation rate, and nitric oxide treatment produced a further increase in mutation yield. Sequence analysis showed that methylation alone led to C:G - T:A transitions, whereas nitric oxide treatment simply produced more C:G - A:T transversions. Thus the most important factor in C:G - T:A transition at CpG sites identified in this experimental system is cytosine methylation, consistent with spontaneous conversion of 5-methylcytosine to thymine by deamination.

Murata-Kamiya N, Tsutsui T, Fujino A, Kasai H, Kaji H. **Determination of carcinogenic potential of mineral fibers by 8-hydroxydeoxyguanosine as a marker of oxidative DNA damage in mammalian cells.** Int Arch Occup Environ Health 1997;70(5):321-6.
8-Hydroxydeoxyguanosine (8-OH-dG) is a typical form of oxidative DNA damage, which causes mutations in vitro and in vivo. To develop a simple method of testing the carcinogenicity of fibrous materials, the formation of 8-OH-dG was determined in the DNA of J774 cells, an established reticulum cell sarcoma line, after treatment with various natural and man-made mineral fibers. The amount of 8-OH-dG was determined using high-pressure liquid chromatography (HPLC) equipped with an electrochemical detector (ECD). We tested three natural mineral fibers (crocidolite, amosite, and chrysotile) and three man-made mineral fibers (ceramic, glass, and potassium octatitanate). Among them, a significant increase in 8-OH-dG formation was observed in the crocidolite- and amosite-treated cells. We also measured the amount of tumor necrosis factor (TNF) produced by J774 cells incubated with the fibrous materials. Cellular TNF production increased after treatment with all the fibers tested, but it was not statistically significant except in the case of chrysotile. Therefore, these results indicate that the mechanism of TNF production is different from that of 8-OH-dG formation, and that the carcinogenicity of various fibrous materials can be better evaluated by measuring the 8-OH-dG level in J774 cellular DNA after treatment with these fibers.

Nelson E. **Laboratory probing of oncogenes from human liquid and solid specimens as markers of exposure to toxicants**. CRC Crit Rev Toxicol 1996;26(5):483-549.
The suitability of oncogenes such as p53 and ras as markers of exposure to toxicants in occupational settings was discussed. The role of the tumor suppressor gene, p53, in oncogenesis was described in the context of occupational exposures to heavy metals and chlorine compounds. Laboratory detection of p53 was discussed, including techniques such as immunohistochemical analysis, loss of heterozygosity assay, single strand conformation polymorphism analysis, flow cytometry, constant denaturant gel electrophoresis, and restriction fragment length polymorphism analysis. The role of ras oncogenes in cancer markers was also described, and the detection of ras oncogenes in human cancers was presented in a table. The relationship between occupational exposure and ras was discussed. Laboratory detection of ras included immunoblotting analysis, dot blot screening, cleavage of single base mismatches, and restriction site mutation analysis. The use of animals models in the determination of oncogenicity was discussed. There has been increasing evidence linking chemical specific changes of the p53 gene with most human cancers. The author concludes that detection of changes in p53 and ras as biomarkers of toxicant exposure in humans in occupational settings would serve as a reliable method of detection.

Okamoto M, **Simultaneous demonstration of lens regeneration from dorsal iris and tumour**

production from ventral iris in the same newt eye after carcinogen administration. Differentiation 1997;61(5):285-92.
BIOSIS COPYRIGHT: BIOL ABS. It is well known that urodeles have the most powerful regenerative capacities among vertebrates, but there is little realisation that they are extremely resistant to spontaneous or chemically induced tumours. Regeneration and carcinogenesis have been considered to be two sides of the same mechanism. Since antagonism between regeneration and carcinogenesis was expected in previous studies, the present study was intended to clarify this relationship in greater detail by changing the amounts of carcinogen stepwise. When 1 mul nickel subsulfide solution was administered in various amounts (1 mug/pl 40 mug/mul) into lentectomized newt eyes, the delay of initiation in lens regeneration for 6 months and an increased inhibition rate of lens regeneration at 6 months were observed in proportion to the increase in carcinogen dosage. The tumour production rate increased in accordance with the increase in the amounts of carcinogen. The conspicuous result obtained in the present study was that lens regeneration from dorsal iris and tumour induction from ventral iris occurred simultaneously in the same eye after administration of moderate amounts (10 mug/mul) of carcinogen. These data clearly indicated that the regenerating dorsal iris is persistently resistant to carcinogen, whereas the ventral iris, which cannot regenerate lens, is susceptible to tumour induction. This strongly suggests that the lens regeneration system in the newt has special advantages for research on the relationship between regeneration and carcinogenesis.

Peters JM, Cattley RC, Gonzalez FJ. **Role of PPAR alpha in the mechanism of action of the nongenotoxic carcinogen and peroxisome proliferator Wy-14,643.** Carcinogenesis 1997;18(11):2029-33.
Chronic administration of peroxisome proliferators to mice and rats results in hepatomegaly and ultimately carcinogenesis. The mechanism underlying the carcinogenic effect of nongenotoxic peroxisome proliferators is not well understood. To determine whether nongenotoxic carcinogenesis is receptor mediated, we evaluated the effect of the prototypical peroxisome proliferator Wy-14,643 on replicative DNA synthesis and carcinogenesis in the PPAR alpha-null mouse line. Male mice (F4, Sv/129 ter) of both genotypes (+/+) and (-/-) were fed either a control diet or one containing 0.1% Wy-14,643 for either 1 week, 5 weeks, or 11 months. Wild-type mice fed the Wy-14,643 diet for 1 or 5 weeks showed increased hepatic labeling by bromodeoxyuridine (BrDU) compared to untreated controls. In contrast, there was no increase in hepatic BrDU labeling index in (-/-) mice fed the Wy-14,643 diet for the same time periods compared to controls. After 11 months, 100% of the (+/+) mice fed the Wy-14,643 diet had multiple hepatocellular neoplasms, including adenomas and carcinomas, while the (-/-) mice fed the Wy-14,643 diet were unaffected. This work demonstrates that the effects of Wy-14,643 on replicative DNA synthesis and hepatocarcinogenesis are mediated by PPAR alpha.

Rivera JA, Graeme-Cook F, Werner J, Z'graggen K, Rustgi AK, Rattner DW, Warshaw AL, Fernandez-Del Castillo C. **A rat model of pancreatic ductal adenocarcinoma: targeting chemical carcinogens.** Surgery 1997;122(1):82-90.
BIOSIS COPYRIGHT: BIOL ABS. Background. Current experimental models of pancreatic cancer either fail to reproduce the ductal phenotype or cause simultaneous cancers in other organs also. To develop an animal model of pancreatic cancer that accurately mimics the human condition, we restricted carcinogenic exposure to the pancreas and specifically targeted ductal epithelial cells. Three different

carcinogens were either implanted directly into the pancreas or infused into the pancreatic duct, with or without near-total pancreatectomy (as a means of inducing pancreatic ductal cell proliferation). Methods. Groups of male Sprague-Dawley rats were exposed to varying doses of dimethylbenzanthracene (DMBA), methynitronitrosoguanidine, or ethylnitronitrosoguanidine either through direct implantation into the pancreas or infusion into the pancreatic duct. Near-total pancreatectomy was added in all groups except two DMBA implantation groups. Surviving rats were killed at 3, 6, 9, or 12 months, and the pancreata were evaluated histologically. Results. All three carcinogens caused pancreatic inflammation, ductal hyperplasia, atypia, and dysplasia beginning by 3 months and becoming more prominent at later time points. Only DMBA caused frequent invasive pancreatic ductal adenocarcinoma, which was first evident by 6 months. The prevalence of pancreatic cancer among DMBA-treated rats evaluated after 10 months was 39% (19 of 49). The addition of pancreatic resection did not enhance pancreatic cancer development. Conclusions. Of the strategies tested, only direct implantation of DMBA into the rat pancreas frequently produces pancreatic cancer histologically similar to human ductal adenocarcinoma. The development of hyperplastic, atypical, and dysplastic changes preceding and accompanying carcinomas suggests that these lesions are preneoplastic. This model recapitulates the progression from normal to neoplastic epithelium and is likely to be useful for the study of morphologic and molecular mechanisms underlying the early stages of pancreatic carcinogenesis and for the investigation of novel diagnostic and therapeutic techniques.

Ruder AM. **Epidemiology of occupational carcinogens and mutagens**. Occup Med 1996;11(3):487-512.
Epidemiological issues related to occupational carcinogens and mutagens were reviewed. A search was conducted in the Registry of Toxic Effects of Chemical Substances database; 654 substances were discovered which had reviews from the International Agency for Research on Cancer (IARC) and exposure estimates from either the National Occupational Exposure Survey or the National Occupational Hazard Survey. The IARC's rating system, in which carcinogens were classified according to evidence of carcinogenicity as sufficient, limited, insufficient, and lack of evidence, was discussed. Although epidemiological studies were considered useful to the study of occupational carcinogenicity, in-vitro mutagen assays were regarded as the more efficient and cost effective study type. Based on the modified Ames test assay, most animal carcinogens were found to be mutagenic. However, bacterial mutagenicity was not viewed as a consistent indicator of mammalian mutagenicity. Determining the mutagenicity of a substance was considered important not only because of mutagenic effects, but also because of the link between mutagenicity and carcinogenicity. Several mutagenicity tests were reviewed, such as tests which used human peripheral lymphocyte cells as the substrate. Other pertinent issues to mutagenicity tests, such as confounding, choice of the referent group, and utility in large scale screening programs, were also addressed. The author concludes that further study of mutagenicity and carcinogenicity is crucial, since many workers are exposed to thousands of unevaluated chemicals. More epidemiological studies are also needed to better classify the substances currently listed as possible and probable carcinogens.

Sanner T, Dybing E, Kroese D, Roelfzema H, Hardeng S. **Potency grading in carcinogen classification.** Mol Carcinog 1997;20(3):280-7.
In 1992 the United Nations Conference on Environment and Development decided to harmonize

carcinogen classification systems. A proposal for a harmonized classification system is currently being considered by the Organization for Economic Cooperation and Development (OECD). In many countries, classification of a chemical as carcinogenic triggers labeling requirements. Implicit in the labeling requirements are often restrictions on the sale of consumer products and workplace regulations. Many of the current classification systems for carcinogens use a single concentration limit for the minimum concentration of a carcinogen in a preparation (mixture) that requires labeling. For high-potency carcinogens, one concentration limit may not adequately express the hazard, whereas for low-potency carcinogens, one limit may overestimate the hazard caused by the carcinogen in the preparation (mixture). The potency grading system discussed consists of three potency groups: high-, medium-, and low-potency carcinogens. It is envisioned that the different classes will trigger different labeling requirements. In the process of potency grading, a preliminary conclusion as to whether a substance shows high, medium, or low potency is initially based on a tumorigenic dose descriptor. The preliminary potency evaluation may then be modified after due consideration of a number of additional elements. These may include evaluation of the dose-response curve; site-, species-, strain-, and sex-specific activity; mechanisms including genotoxicity; mechanistic relevance to humans; toxicokinetics; and other factors. The potency grading system discussed is applicable to most carcinogen classification systems, including that currently being considered by the OECD.

Satoh K, Sakagami H, Kadofuku T, Kurihara T, Motohashi N. **Radical intensity and carcinogenic activity of benz[c]acridines.** Anticancer Res 1997;17(5a):3553-7.
Among 13 benz[c]acridines, six 7-methyl-substituted compounds (7-methylbenz[c]acridine, 7,9-dimethylbenz[c]acridine, 7,10-dimethylbenz[c]acridine, 7,11-dimethylbenz[c]acridine, 7,9,10-trimethylbenz[c]acridine, 7,9,11-trimethylbenz[c]acridine) were carcinogenic, while the other seven compounds (benz[c] acridine, 8-methylbenz[c]acridine, 9-methylbenz[c]acridine, 10-methylbenz[c] acridine, 11-methylbenz[c]acridine, 5,7-dimethylbenz[c]acridine, cis-5,6-dihydroxy-5,6-dihydrobenz[c] acridine) were inactive. Using both McLachlan-Huckel molecular orbital (McLachlan-HMO) and HMO methods, all the carcinogenic compounds were shown to have the elevated pi-spin density at 12th nitrogen atom of their molecules, as compared with noncarcinogenic compounds. Electron spin resonance (ESR) spectroscopy, however, revealed that both carcinogenic and noncarcinogenic compounds produced no detectable amounts of radical. This is in contrast to ascorbates, gallates and benzo[a]phenothiazines, which induced apoptosis by radical mediated mechanism(s). Amino acid analysis demonstrated that methionine oxidation is not involved in the induction of carcinogenic activity by benz[c]acridines.

Shirai T. **A medium-term rat liver bioassay as a rapid in vitro test for carcinogenic potential: a historical review of model development and summary of results from 291 tests.** Toxicol Pathol 1997;25(5):453-60.
BIOSIS COPYRIGHT: BIOL ABS. A bioassay system for rapid detection of carcinogenic agents has been developed using male Fischer 344 rats to bridge the gap between long-term carcinogenicity tests and short-term screening assays. The system, called the medium-term liver bioassay, is fundamentally based on the 2-stage hypothesis of tumor production, employing initiation by diethylnitrosamine (200 mg/kg, ip) in the first stage and test chemical administration during the second, in combination with two-thirds partial hepatectomy. It requires only 8 wk for animal experimentation and a further few weeks for

quantitative analysis of immunohistochemically demonstrated glutathione S-transferase placental form positive hepatic foci. A total of 291 chemicals/substances have already been analyzed in our laboratory. Among 63 chemicals that were proved to be carcinogenic in the liver of rat and/or mouse, 57 (90%) gave positive results irrespective of their mutagenicity. Negative compounds include peroxisome proliferators and tamoxifen. Even nonhepatocarcinogens were positive at a rate of 24%. Eighty-six percent (12/14) of mouse liver carcinogens were also positive. On the other hand, only 2 out of 45 noncarcinogens showed very weak positivity. Thus, the efficacy of the system for hepatocarcinogens has been well established. This bioassay is increasingly regarded as an appropriate alternative test for carcinogenicity risk assessment and is practically used for a rapid evaluation of hepatocarcinogenicity of chemicals.

Siegall CB, Haggerty HG, Warner GL, Chace D, Mixan B, Linsley PS, Davidson T. **Prevention of immunotoxin-induced immunogenicity by coadministration with CTLA4Ig enhances antitumor efficacy.** J Immunol 1997;159(10):5168-73.
Immunotoxins have shown promise as antitumor agents in clinical trials. However, they have not become part of standard cancer therapy because of factors that include their inherent immunogenicity, which limits the duration of therapy. To address this issue, we evaluated in preclinical models the concomitant use of the immunosuppressive agent CTLA4Ig and BR96 sFv-PE40, a single-chain immunotoxin that binds to carcinoma cells expressing Le(y). Cotreatment with CTLA4Ig, an inhibitor of the CD28/CTLA4-CD80/CD86 costimulation pathway, blocked the production of Abs against BR96 sFv-PE40 in immunocompetent rodents and dogs. It also blocked hypersensitivity reactions in rats carrying colon carcinoma allografts during a second course of BR96 sFv-PE40 therapy, and the cotreatment with CTLA4Ig resulted in enhanced antitumor activity. Cotreatment with CTLA4Ig also prevented hypersensitivity reactions induced by repeat dosing of BR96 sFv-PE40 (q3dx5) in dogs. The production of anti-BR96-sFv-PE40 Abs was decreased in CTLA4Ig-cotreated rodents and dogs resulting in increased plasma levels of BR96 sFv-PE40 relative to non-CTLA4Ig-cotreated animals. These data show that cotreatment of immunotoxins with CTLA4Ig, by inhibiting the production of anti-immunotoxin Abs, can extend the duration of BR96 sFv-PE40 therapy to give greater exposure, reduced toxicities, and increased efficacy.

Sjogren M, Ehrenberg L, Rannug U. **Relevance of different biological assays in assessing initiating and promoting properties of polycyclic aromatic hydrocarbons with respect to carcinogenic potency**. Mutat Res 1996;358(1):97-112.
The underlying mechanisms of carcinogenesis were examined at specific dose levels using relevant mechanistic and mathematic models. A secondary aim was to identify the initiating and promoting properties responsible for the carcinogenicity of polycyclic aromatic hydrocarbons (PAH). Altogether, 29 PAHs were included in the study. Biological effects were assayed using 14 variables, including bacterial mutagenicity, enhancement and inhibition of bacterial mutagenicity, Ah receptor affinity, and induction of enzymes involved in the bioactivation of PAH compounds to mutagens. The highest correlations with cancer data were observed for variables describing Ah receptor (AhR) affinity, whereas bacterial mutagenicity data were poorly correlated with cancer data as determined by a principal components analysis. Only one bacterial mutagenicity variable, but all AhR affinity variables were statistically relevant to describe carcinogenic potency as determined by a partial least squares

regression analysis. A correlation was also noted with the latter variables and the inhibition of bacterial mutagenicity of benzo(a)pyrene (50328). The authors conclude that structural requirements for AhR affinity are the same as those needed for metabolism by enzymes that bioactivate benzo(a)pyrene. A negative correlation was noted between mutagenicity and induction of enzymes. The authors propose that bacterial mutagenicity effects the cancer initiation potency, while the AhR affinity reflects the promotive effect of some PAH at the high doses applied in rodent carcinogenicity tests. They note that initiation and promotion are provoked by different chemical species, these being reactive metabolites and the parent hydrocarbons, respectively. The effects of initiation may be more important in the course of chemical carcinogenesis at doses reflecting a normal human exposure.

Sundberg JP, Sundberg BA, Beamer WC. **Comparison of chemical carcinogen skin tumor induction efficacy in inbred, mutant, and hybrid strains of mice: morphologic variations of induced tumors and absence of a papillomavirus cocarcinogen.** Mol Carcinog 1997;20(1):19-32.
BIOSIS COPYRIGHT: BIOL ABS. Chemical carcinogen induction of skin tumors in mice was investigated to determine (i) if tumor induction efficacy was modified by single gene mutations, (ii) if the histologic types of the tumors varied with these mutations, and (iii) if a novel papillomavirus was involved as a cocarcinogen. A two-stage carcinogenesis protocol (7,12-dimethylbenz(a)anthracene followed by 12-O-tetradecanoylphorbol-13-acetate) was used to induce papillomas in 14 inbred, two hybrid, and 15 other genetic stocks of mice with inherited, single-gene mutations causing skin abnormalities. Histopathological, immunohistochemical, and Southern blot analyses were performed to determine tumor type and to detect the presence of papillomaviruses. The histologic types of tumors induced included early follicular papillomas, mixed papillomas, exophytic papillomas, hyperplastic papillomas, fibropapillomas, squamous cell carcinomas, and mast cell tumors. The efficacy of tumor induction was influenced by strain background, as seen by the clustering of mice into high-, intermediate-, and non-responding groups. Similarly, tumor induction efficacy was affected by specific mutant genes that cause skin abnormalities. No evidence of papillomavirus structural antigens or viral genomic DNA was identified in 547 induced tumors. These observations indicate that numerous modifier genes but not papillomaviruses are involved in cutaneous chemical carcinogenesis.

Warren AJ, Shields PG. **Molecular epidemiology: carcinogen-DNA adducts and genetic susceptibility.** Proc Soc Exp Biol Med 1997;216(2):172-80.
BIOSIS COPYRIGHT: BIOL ABS. Molecular epidemiological studies assess individual chemical exposures and genetic susceptibility in order to identify cancer risk. Such studies incorporate the development, application, and validation of biomarkers of cancer risk in order to enhance cancer risk assessments, focus cancer prevention strategies, and elucidate mechanisms of carcinogenesis. Current studies of molecular epidemiology are based upon an understanding of the complex, multistage process of carcinogenesis and interindividual variations in response to carcinogenic exposures. Quantitative methods to measure human exposures to carcinogens continue to improve and have been successfully applied to a number of epidemiological studies. Genetic predispositions to cancer, both inherited and acquired, have been and continue to be identified. The combined approach of associating genetic polymorphisms with carcinogen-DNA adduct measurements, in order to assess cancer risk, is showing considerable promise. It is hoped that, in the future, molecular epidemiologists will be able to develop a risk profile for an individual that includes assessment of multiple biomarkers. The field has the near-

term potential to have a significant impact on regulatory quantitative risk assessments, which may aid in the determination of allowable exposures. Molecular epidemiological data may also aid in the identification of individuals who will most benefit by cancer prevention strategies.

Wild CP, Pisani P. **Carcinogen-DNA and carcinogen-protein adducts in molecular epidemiology.** IARC Sci Publ 1997;142:143-58.
Carcinogen-DNA and carcinogen-protein adducts provide an integrated measure of carcinogen exposure, uptake and absorption, metabolism, DNA repair and cell turnover. As such they promise to provide a more objective and relevant measure of exposure than that which can be derived from questionnaires and measures of ambient levels of carcinogen. Nevertheless, the interpretation of adduct measurements made in human tissues and body fluids requires an understanding of a number of factors. These include the sensitivity and specificity of the measurement, the temporal relationship between exposure and adduct level and the mechanistic role of the adduct in the process of carcinogenesis. The application of such biomarkers in epidemiological studies therefore necessitates careful consideration of optimal study design. The above issues are illustrated in this chapter with examples from studies in both animal models and human populations.

CYTOTOXICITY

Feng Y, Fretland AJ, Rustan TD, Jiang W, Becker WK, Hein DW. **Higher frequency of aberrant crypt foci in rapid than slow acetylator inbred rats administered the colon carcinogen 3,2'-dimethyl-4-aminobiphenyl.** Toxicol Appl Pharmacol 1997;147(1):56-62.
Humans and other mammals such as rats exhibit a genetic polymorphism in acetyltransferase (NAT2) capacity, yielding rapid and slow acetylator phenotypes. The rapid acetylator phenotype has been associated with increased incidence of human colorectal cancer in some, but not all, epidemiological studies. In order to investigate this possible association, a rapid (F-344) and slow (WKY) acetylator inbred rat model was utilized to investigate the role of the acetylator genotype (NAT2) in the formation of aberrant crypt foci (ACF) following administration of colon carcinogens. Age-matched (retired breeder) female rapid and slow acetylator inbred rats received two weekly injections (50 or 100 mg/kg, sc) of 3,2'-dimethyl-4-aminobiphenyl (DMABP) or a single 50 mg/kg, sc, injection of 1,2-dimethyl-hydrazine (DMH). The rats were euthanized at 10 weeks and ACF were evaluated in the cecum, ascending, transverse, and descending colon, and rectum. ACF were observed in the colon and rectum, but not the cecum of rapid and slow acetylator inbred rats administered DMABP or DMH. ACF were more concentrated in the descending colon. ACF frequencies were significantly higher in colons of rapid than slow acetylator inbred rats administered DMABP, a colon carcinogen which is activated via O-acetylation catalyzed by polymorphic acetyltransferase (NAT2). At 50 mg/kg, ACF frequency in the distal colon was 2.29 +/- 0.57 in rapid acetylators versus 0.38 +/- 0.18 in slow acetylators. At 100 mg/kg, ACF frequency was 4.11 +/- 1.06 in rapid versus 1.57 +/- 0.48 in slow acetylators. ACF frequency did not differ significantly between rapid and slow acetylator inbred rats administered DMH, a colon carcinogen which is not metabolized by polymorphic acetyltransferase. The two inbred rat strains did not differ in hepatic microsomal phenacetin deethylase activity, which is a marker for CYP1A2 activity important for the activation of aromatic amines. These results support the hypothesis that rapid acetylator (NAT2) genotype is a risk factor in aromatic amine-induced colon carcinogenesis. Copyright

Fratello G, Marchini S, Zucco F, Sapora O, Stammati A. **Cytotoxicity of halogenated benzenes and its relationship with logP.** Toxicol In Vitro 1997;11(5):673-7.
 The in vitro toxicity of a series of environmentally relevant halobenzenes was tested using a Chinese hamster lung fibroblast cell line and its relationship with the logarithm of octanol/water partition coefficient (logP) was investigated. Since we wanted to study the direct biological activity of the parent substances, we have used the V-79 cell line that does not express phase I metabolic activities. Moreover, because of the available knowledge on the substances, we decided to perform the colony-forming ability test (CFA) and to analyse the DNA damage by a cytofluorimetric assay. To identify the concentration range at which the toxic effect could be detected, a prescreen with the neutral red assay has been performed. All the substances tested were positive in the CFA, but, according to the concentration values inhibiting this activity by 50%, they can be divided into two groups of differing toxicity. The FACS can analysis shows for the majority of the halobenzenes a clear hypodiploid peak. A good correlation between values describing the concentration that inhibits CFA by 50% and logP was found, indicating that it depends on the hydrophobic properties of the compounds and that logP is a suitable descriptor.

Huveneers-Oorsprong M B, Hoogenboom L A, Kuiper HA. **The use of the MTT test for determining the cytotoxicity of veterinary drugs in pig hepatocytes.** Toxicol In Vitro 1997;11(4):385-92.
 Pig hepatocytes were used for determining the cytotoxicity of a number of veterinary drugs and known hepatotoxic compounds, using the MTT test as a marker for viability. When possible, drugs were tested in the absence and presence of dimethyl sulfoxide (DMSO), to study the possible effect of this solvent when used in the case of less hydrophilic compounds. IC50 values calculated from the dose-response curves for acetylsalicylic acid (7 mM) and acetaminophen (paracetamol; 10 mM) in rat hepatocytes were similar to those reported by other groups. IC50 values for acetylsalicylic acid (8.7 mM), erythromycin (0.7 mM), chloramphenicol (8.1 mM), stilboestrol (diethylstilbestrol; 0.16 mM and propranolol (0.17 mM) in pig hepatocytes were similar to those reported in the literature for rat hepatocytes. In comparison to rat hepatocytes, clenbuterol was about equally cytotoxic in pig hepatocytes (IC50 of 2.1 v. 1.6 mM), whereas paracetamol was much more cytotoxic (IC50 of 2.8 v. 10 mM). Unlike chloramphenicol, the related drug thiamphenicol showed no signs of decreased MTT formation in pig hepatocytes at the highest possible test concentration of 10 mM, as was the case for furazolidone, oxytetracycline, carbadox and the putative furazolidone and furaltadone metabolites 3-amino-2-oxazolidinone and 3-amino-5-morpholinomethyl-2-oxazolidinone tested at concentrations up to (respectively) 500 muM, 3 mM, 100 muM, 5 mM and 5 mM. IC50 values of 22 muM and 0.25 mM were obtained for menadione and furaltadone, respectively. DMSO, used at a concentration of 1%, had no effect on the toxicity of acetylsalicylic acid, erythromycin, propranolol and clenbuterol in pig hepatocytes. In the case of acetaminophen, DMSO significantly reduced its toxicity in pig hepatocytes (IC50 of 5.1 v. 2.8 mM), but not in rat hepatocytes. DMSO also significantly reduced the cytotoxicity of furaltadone in pig hepatocytes (IC50 of 0.87 v. 0.25 mM). Following incubation with 0.5 mM furaltadone in the absence of 1% DMSO, intracellular GSH levels were decreased (38 v. 49 nmol/mg protein), whereas in the presence of DMSO a slight increase (59 v. 52 nmol/mg protein) was observed. DMSO had no effect on the overall degradation of the related drug furazolidone, or the

formation of protein-bound metabolites. It is hypothesized that DMSO is involved in the detoxification of reactive oxygen species generated during the degradation of nitrofuran drugs, either directly or through a stimulation of the synthesis of glutathione. It is concluded that pig hepatocytes are a valuable toot to study the cytotoxicity of veterinary drugs and possible interactions with other xenobiotics, and to reveal possible species differences between farm animals and laboratory animals used to study the toxicology of these compounds.

Maynard RL, Cameron KM, Fielder R, Mcdonald A, Wadge A. **Setting air quality standards for carcinogens: an alternative to mathematical quantitative risk assessment-discussion paper.** Regul Toxicol Pharmacol 1997;26(1 Pt 2):60-70.
It has been accepted in many countries that the regulation of ambient air quality should involve the use of health-based air quality standards. Setting standards for air pollutants which are genotoxic carcinogens presents difficult problems to the regulator, in that the prediction of the effects on health of low levels of exposure is suspected to be inaccurate, and is not currently amenable to either experimental or epidemiological verification. In some countries, techniques of mathematical quantitative risk assessment have been adopted to calculate acceptable levels of exposure to, or the unit risk factors for, genotoxic carcinogens. We regard these approaches as unsatisfactory. An alternative approach, based upon a number of argued premises, a strategy which identifies decision points and the cautious application of uncertainty factors, is described. Copyright 1997 Academic Press.

Patel HR, Hewer A, Phillips DH, Hayes JD, Wolf CR, Campbell FC. **Metabolic competence and susceptibility of intestinal epithelium to genotoxic injury during regeneration.** Carcinogenesis 1997;18(11):2171-7.
The carcinogenic potency of many mutagens is increased in conditions of tissue regeneration. This involves fundamental changes of cellular division and differentiation, in intestinal epithelium. However, effects on epithelial capacity for carcinogen metabolism and susceptibility to genotoxic injury are unknown. Using a novel rat model, this study assessed expression of cytochrome P450 mono-oxygenases (Cyps), glutathione S-transferases (GSTs) and uridine diphosphoglucuronosyl transferase (UGT) in intestinal epithelium during sequential stages of regeneration. Enzyme induction and DNA adduct formation were also assessed after benzo[a]pyrene (BaP) exposure. Control assays were carried out in normal intestinal epithelium. Fewer phase I and II xenobiotic metabolizing enzymes were expressed in regenerating intestinal epithelium than in normal control intestinal epithelium (GSTA3, UGT in regeneration vs Cyp2B, GSTA1/2, GSTA4, GSTP1, UGT in control). Benzo[a]pyrene induced GSTA3 and UGT in regeneration vs Cyp1A, Cyp2B, GSTA1/2, GSTA3, GSTA4, GSTP1 and UGT in control normal intestinal epithelium. Benzo[a]pyrene induced low levels of GSTA3 in early regenerating intestinal epithelium but induction increased by >2-fold at late stage regeneration. Higher levels of benzo [a]pyrene 7,8-diol-9,10-epoxide (BPDE) DNA adducts were formed at early stages of regeneration, than at later stages. Intestinal epithelium displayed reduced metabolic competence and differential susceptibility to genotoxic injury from BaP, during regeneration.

Qiao L, Hanif R, Sphicas E, Shiff SJ, Rigas B. **Effect of aspirin on induction of apoptosis in HT-29 human colon adenocarcinoma cells.** Biochem Pharmacol 1998;55(1):53-64.
Aspirin (ASA) and other nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit colorectal

tumorigenesis. Apoptosis is a critical determinant of tissue mass homeostasis and may play a role in carcinogenesis. We studied the effect of ASA on the survival of a human colon cancer cell line using more sensitive methods than we had applied previously. ASA induced apoptosis in HT-29 colon adenocarcinoma cells at concentrations > or =1 mM as established by: (a) morphological changes consistent with apoptosis in cells examined by fluorescence microscopy and semi-thin cell sections, and (b) DNA strand breaks: 45% of the cells were TdT-mediated dUTP nick end labeling (TUNEL) positive at 3 mM at 72 hr, and 70% were positive by the comet assay. Electron microscopy also confirmed the induction of apoptosis by ASA. ASA-induced apoptosis was not associated with: (a) a ladder pattern on genomic DNA electrophoresis, or (b) a subdiploid peak on flow cytometry. Apoptotic bodies were virtually absent on standard morphological assessments and only a few were detected on semi-thin sections. For the above reasons, this apoptosis induced by ASA is atypical, and the unusual features of ASA-induced apoptosis, besides their taxonomic value, may offer clues to the mechanisms that control the process of apoptosis or perhaps the cancer chemopreventive properties of this compound. These findings demonstrate that ASA induces apoptosis in human colon cancer cells, bolstering the hypothesis that apoptosis may be a mechanism by which NSAIDs inhibit colon carcinogenesis. These findings should be examined in animal and/or clinical research studies in vivo.

Torricelli A, Bisiach M, Spinelli L, Ubezio P. **From flow cytometric BrdUrd data to cell population growth and doubling time.** Cytometry 1997;29(3):222-32.
We describe a direct way to use flow cytometric data for measuring the growth curve of a cell population. The starting point is analysis of the intrinsic informative content of the time course, after bromodeoxyuridine (BrdUrd) labeling, of the percentages of cells detected within four windows of biparametric BrdUrd-DNA histograms. We did not introduce a particular cell cycle model or use the hypothesis of exponential growth. We obtained a simple formal proof of the existence of four independent formulae connecting the flow cytometric data and the relative growth curve of the cell population. The formulae were then challenged in a number of simulated kinetic scenarios, moving away from their expected limits of validity. The results suggest additional uses of the formulae and a way of estimating cell-cycle-phase durations. Considering exponential growth in the presence of cell loss, the formulae were used to estimate the potential doubling time from a single flow cytometric measure vs. other procedures that additionally require an estimate of the duration of the phase S. The theoretical precision of the procedures may differ depending on how cell loss occurs.

Zange R, Kissel T. **Comparative in vitro biocompatibility testing of polycyanoacrylates and poly(D, L-lactide-co-glycolide) using different mouse fibroblast (L929) biocompatibility test models**. Eur J Pharm Biopharm 1997;44(2):149-57.
CBAC COPYRIGHT: CHEM ABS Comparative in vitro cytotoxicity studies of polyalkylcyanoacrylates (PCA) and poly(D,L-lactide-co-glycolide) (PLGA) were performed. Four PCAs of different alkyl chain lengths and 2 com. available PLGAs were assayed in several cell culture models recommended by the International Std. Organization (ISO). Different polymer prepns., 2 extn. methods evaluated by the MTT (3-(4,5-dimethylthiazo1-2-yl)-2,5-diphenyl tetrazolium bromide) assay and LDH (lactate dehydrogenase) release, 2 indirect contact methods, for example the agar overlay, and a direct contact method were used. Test procedures included light microscopy, the vital stain neutral red, the MTT assay, based on the cleavage of a yellow tetrazolium salt (MTT) to purple formazan crystals by mitochondrial

active cells, LDH release and SEM. All polycyanoacrylates tested showed cytotoxic effects with methylcyanoacrylate being the most toxic compd. Cytotoxic effects and proliferation inhibition decreased with increasing alkyl chain length of the PCAs. PLGA was very well tolerated by the cultured cells in all assays performed. The extn. method evaluated by the MTT assay seems to be the most appropriate method, as it showed a clear graduation of toxicity and allowed an estn. of the 50% inhibitory concn. (IC50) values for all polymers tested.

Zizka Z, Pelc R. **Quantitative assessment of cell damage in situ: electron microprobe X-ray analysis of model organisms treated with noxious species.** Folia Microbiol 1997;42(5):530-2.
BIOSIS COPYRIGHT: BIOL ABS. The existing set of methods for assessing toxicity of noxas, based on experiments with whole animals (subclinical toxicity, toxicokinetics, carcinogenity, teratogenity, neurotoxicology etc.) does not provide much information about cellular and subcellular effects such compounds may exert. We suggest to complement the current methodology by combining a traditional morphological observation in an electron microscope with a spectroscopic method of electron microprobe X-ray analysis (or X-ray microanalysis). The latter makes it possible to measure concentrations of chemical elements in individual cells and organelles and effects of noxas can thus be assessed (i) at subcellular level, (ii) directly in situ and (iii) quantitatively. Concentrations of biologically important elements such as phosphorus, sulfur or zinc were measured in individual organelles in both intact and noxa-treated tissues, thus offering a possibility of comparing the effects of various noxious species at subcellular level (with the noxa previously applied to whole tissue or animal). The suggested correlation of analytical and morphological information may also provide new insights into cellular targeting of noxas (and potentially also drugs) as some organelles appear to be much more susceptible to damage than others.

DERMAL TOXICITY

Augustin C, Collombel C, Damour O. **Use of dermal equivalent and skin equivalent models for identifying phototoxic compounds in vitro.** Photodermatol Photoimmunol Photomed 1997;13(1-2):27-36.
Phototoxicity inducing in vivo photoirritation, a reversible inflammatory reaction of the skin after chemical contact and UVA radiation exposure, is increasingly observed as a side effect associated with the use of both cosmetics and systemic drugs. In order to systematically screen for the phototoxic potential of new compounds, we propose two three-dimensional models suitable for in vitro testing: a dermal equivalent (DE) and a skin equivalent (SE) model. The DE model includes a collagen-glycosaminoglycans-chitosan porous matrix populated by normal human fibroblasts. The SE model is made by seeding normal human keratinocytes onto the DE, leading to a fully differentiated epidermis. The objectives of this pilot study are: 1) to compare the deleterious effects of UVA radiation on the two models and 2) to evaluate to what extent the in vitro results can predict the in vivo phototoxicity caused by well-known photoirritant compounds, included in the COLIPA validation phototoxicity reference chemical list. Dilutions of thiourea, sulisobenzone, promethazine, chlorpromazine and tetracycline were applied (20 microliters) onto DEs and SEs (n = 6) and incubated for 1 h (or 15 h) at 37 degrees C. Irradiated samples received 3 J/cm2 UVA. The 24 h post-irradiation residual cellular viability was measured using the MTT test on treated and untreated tissues and IL-1 alpha release measurement in

collected SE culture media. A concordance in terms of photoirritant/non-photoirritant was obtained between the in vivo data and the in vitro results, suggesting that the DE and the SE models could be integrated, after a complete validation study, into a protocol for in vitro testing of the photoirritant potential of new molecules.

Basketter DA, Chamberlain M, Griffiths HA, Rowson M, Whittle E, York M. **The classification of skin irritants by human patch test.** Food Chem Toxicol 1997;35(8):845-52.
The human 4 hour patch test provides an opportunity to identify substances with significant skin irritation potential without recourse to the use of animals. The protocol is designed to avoid the production of more than mild irritant reactions and meets the highest ethical standards. This paper provides the background to the development of the method and comments on its performance in the light of recent intra- and inter-laboratory investigations. In particular, the value of the method in providing 'gold standard' data for the identification of those substances (or preparations) which should, or should not, be classified as irritant to skin in European legislation is discussed. On the basis of the published data and supplementary investigations, recommendations are made on both the conduct and interpretation of the human 4 hour patch test. Finally, the lack of any necessity for formal validation of this assay is addressed.

Basketter DA, Reynolds FS, York M. **Predictive testing in contact dermatitis. Irritant dermatitis.** Clin Dermatol 1997;15(4):637-44.

Boelsma E, Tanojo H, Bodde HE, Ponec M. **An in vivo-in vitro study of the use of a human skin equivalent for irritancy screening of fatty acids.** Toxicol In Vitro 1997;11(4):365-76.
BIOSIS COPYRIGHT: BIOL ABS. A human skin equivalent (HSE) consisting of reconstructed epidermis on a fibroblast-populated collagen gel was evaluated as a model for irritancy screening. The irritancy potential of a series of saturated and unsaturated fatty acids was investigated in vivo under short-term exposure conditions using transepidermal water loss (TEWL), laser Doppler velocimetry (LDV) and the penetration of hexyl nicotinate as parameters. The effects of the fatty acids in vitro were studied after topical application on HSE using changes in epidermal morphology, changes in interleukin (IL)-1alpha and interleukin-8 mRNA expression and protein levels, and alterations in activity of plasminogen activators as endpoints. The unsaturated fatty acids increased both TEWL and LDV and elevated IL-1alpha and IL-8 mRNA levels, whereas their effects on protein levels were minimal. In contrast, the saturated fatty acids were not very effective in vivo but induced an increase in IL-la protein levels. The type o fatty acid determines not only the way and the extent of skin barrier modulation, but also the pattern of cell mediator production and release. This study stresses the necessity of investigating multiple endpoints for the characterization of a test compound, in particular when studying mild and moderate irritants.

Braut-Boucher F, Pichon J, Wantyghem J, Muriel MP, Giner M, Font J, Aubery M. **Human keratinocyte models: assessment of cell adhesion and dermotoxicity using fluorescent probes.** Toxicol In Vitro 1997;11(5):601-11.
BIOSIS COPYRIGHT: BIOL ABS. To assess the molecular and cellular events that occur in the skin during biological and pharmaco-toxicological processes, we developed different in vitro models. Two

major systems are described: (1) relatively simple ones such as normal human keratinocytes (NHK) grown in monolayer or continuous culture of spontaneously immortalized keratinocyte cells, the HaCaT cell line. This cell line forms a monolayer and displays the same phenotypic morphology, pattern of differentiation markers as NHK. (2) More complex models such as NHK multilayers differentiated on a synthetic porous membrane. Indeed, NHK grown at the air-liquid interface of culture inserts may undergo epidermal differentiation in 21 days (Noel-Hudson et al., 1995a). Under the same culture conditions, no stratification of the HaCaT cell line was obtained. NHK and/or HaCaT monolayers were used to study the cell surface molecules involved in heterologous cell interactions, and to estimate the cytotoxic effects of different compounds through a sensitive fluorimetric microtitration assay. When cell adhesion was measured with calcein-AM labelled lymphocytes, it appeared that lymphocytes display the same behaviour towards NHK or HaCaT cells. The importance of the activation status of each cell and the involvement of alpha2 and beta3beta1 integrins in lymphocyte-keratinocyte interactions were demonstrated. Likewise cytotoxicity of SDS and DNP was easily and rapidly detected with calcein-AM and Alamar blue probes. Skin models in combination with fluorescent probes offer promising alternatives for assessing cell interactions as well as cytotoxic effects.

Carelli V, Di Colo G, Farusi G, Nannipieri E, Serafini MF. **Human callus as a material to model stratum corneum**. Acta Technol Legis Med 1997;8(1):1-17.
CBAC COPYRIGHT: CHEM ABS The purpose of this study was to verify whether drug partitioning into human callus (HC) can be correlated with drug partitioning into stratum corneum (SC). Findings validate the use of HC, an easily available tissue, as a material to model SC in partitioning expts. Moreover, published human skin permeation coeff. data (as log Kp) for chems. in aq. or non-aq. vehicles were analyzed for a semi-empirical relation with human callus/vehicle partition coeff. (as log Phc,v) and mol. wt. (MW). Although the validity of correlation is limited within the constraints of the data set, results suggest the use of log Phc,v to obtain an est. of the Kp values of penetrants, from non-aq. vehicles, provided the vehicles do not act as penetration enhancers. It is suggested that the enhancing properties of a vehicle which acts by increasing drug partitioning into stratum corneum, may be checked up by measures of drug partition coeff. between human callus, pretreated with the vehicle, and water.

Chesnoy S, Couarraze G. **Physicochemical characterization of iontophoresis modified human stratum corneum: influence of current pretreatment**. Acta Technol Legis Med 1996;7(3):217-22.
CBAC COPYRIGHT: CHEM ABS By the application of an elec. field, iontophoresis strongly enhances the transdermal permeation of ionized drugs. The purpose of this work was to det. the influence of human stratum corneum pretreated by current on the percutaneous penetration of propranolol hydrochloride by iontophoresis. The results show that propranolol iontophoresis fluxes are significantly increased on current pretreated stratum corneum in comparison with normal stratum corneum. The physico-chem. characterization of the samples by x-ray diffraction anal. and Differential Scanning Calorimetry led the authors to assume that, during current pretreatment, pre-existents or new pores are opened into the stratum corneum. These new pathways would improve the percutaneous delivery of propranolol hydrochloride by iontophoresis.

Chesnoy S, Doucet J, Durand D, Couarraze G. **Effect of iontophoresis in combination with ionic enhancers on the lipid structure of the stratum corneum: x-ray diffraction study**. Pharm Res 1996

Oct;13:1581-4.
IPA COPYRIGHT: ASHP The effect of iontophoresis in combination with ionic enhancers such as sodium lauryl sulfate and cetrimonium bromide (hexadecyltrimethylammonium bromide) on the lipid structure of the stratum corneum was investigated using x-ray diffraction. The intercellular lipids were extensively reorganized by iontophoresis. This effect was reinforced when sodium lauryl sulfate was combined with cathodal iontophoresis. In contrast, no effect on the structure of skin lipids was detected with cetrimonium bromide treatment. It was concluded that x-ray diffraction is a powerful technique to characterize the mechanism of action of iontophoresis and penetration enhancers on stratum corneum lipids.

Clarys P, Manou I, Barel AO. **Influence of temperature on irritation in the hand/forearm immersion test.** Contact Dermatitis 1997;36(5):240-3.
As indicated by in vitro experiments the penetration of irritants through the skin is significantly influenced by the temperature of the solution. In vivo experiments, demonstrated equally a significant influence of temperature in surfactant-induced skin irritation. In order to evaluate the irritant potential of detergent solutions under normal user conditions, we used the hand/forearm immersion test. We compared 2 detergents with different anionic character in a repetitive immersion protocol (30 min immersion on 4 consecutive days). The solutions were tested at 2 temperatures (37 degrees C and 40 degrees C). The irritation was quantified by assessment of the stratum corneum barrier function (transepidermal water loss), skin redness (a* colour parameter) and skin dryness (capacitance method). Both detergents affected the integrity of the skin in a significant way. The anionic content as well as the temperature of the solutions were found to be determinative for the irritant potential, with a stronger response for higher anionic content and temperature, respectively.

Deleve LD. **Dinitrochlorobenzene is genotoxic by sister chromatid exchange in human skin fibroblasts**. Mutat Res 1996;371(1-2):105-8.
A study was conducted examining the genotoxicity of dinitrochlorobenzene (25567673) (DNCB) in human skin fibroblasts. The genotoxic effects of DNCB were examined by quantitating sister chromatid exchanges (SCE) and cell growth following in-vitro exposure of biopsy obtained human skin fibroblasts to DNCB. Exposure to 2.5 micromolar or more DNCB induced significant increases in the formation of SCE compared with controls; significantly more SCE were induced following exposure to 7.5 micromolar DNCB. Cell proliferation decreased with increasing concentrations of DNCB; between 99% and 94% of the cells remained following exposure to 2.5 micromolar DNCB, whereas between 76% and 40% of the cells remained following exposure to 10 micromolar DNCB. The implications of these findings to recent suggestions regarding the clinical use of DNCB were discussed.

Dussert AS, Gooris E, Hemmerle J. **Characterization of the mineral content of a physical sunscreen emulsion and its distribution onto human stratum corneum**. Int J Cosmet Sci 1997;19(3):119-29.
IPA COPYRIGHT: ASHP The minerals in a sunscreen water/oil emulsion containing 11% titanium dioxide and 2.5% zinc oxide were characterized, and the distribution of the oxides at the surface of human stratum corneum was studied in vitro. X-ray diffractometry revealed the crystallographic features of the titanium dioxide and zinc oxide crystals. Frequency distributions of crystal length and aspect ratio were determined for zinc oxide, which presented larger particle size and varying shapes. Scanning and

transmission electron microscopy disclosed structural information. A satisfactory mineral distribution along the irregular topography of the skin was observed. No intracellular penetration was noted.

Elton S. **UK research into protection from flame and intense heat for military personnel**. Fire Mater 1996;20(6):293-5.
The philosophy behind research directed at protecting United Kingdom military personnel from the effects of flame and intense heat exposure was discussed. The research philosophy was based on determining in detail the exact nature of the threat presented by fire and intense heat, measuring the performance of current clothing for protecting against the threat, and exploring novel solutions to the problem of protecting personnel against the threat. When considering the nature of fire and heat hazards for military personnel, it was noted that basing protective measures on analyses of temperature events oversimplifies the problem. Temperature measurements alone will not provide information on the rate of heat input experienced by a protective clothing system. It is the rate of heat input that determines how long protection will last. Typical flame or intense heat hazards expected to be experienced by North Atlantic Treaty Organization personnel included burning fuel, exploding ammunition, and penetration by warheads which can produce flux rates on the order of 150, 200, and 500 to 560 kilowatts per minute, respectively. Current research has focused on measuring actual protection levels provided by various clothing ensembles and physiological loading produced among personnel wearing protective clothing ensembles utilizing cone calorimetry and the Hohenstien skin model, respectively. Novel solutions which can provide the desired protection without increasing the thermal load were considered. Two types of novel approaches have been used thus far: clothing that can reflect radiant heat; and reactive clothing that will provide the extra protective insulation at the moment it is needed and not before.

Fang Lihua, Wang Xiasheng. **[The dermato-toxicology study of three kinds of perfumes used in cosmetics]**. Zhonghua Pifuke Zazhi 1997;30(5):318-20. (Chi)
BIOSIS COPYRIGHT: BIOL ABS. Purpose: To find out the mechanism of cosmetic-related dermatitis. Methods: 3 perfumes commonly used in cosmetics were selected (isoeugenol, cinnamic aldehyde and hydroxycitronellal) for laboratory studies of acute skin irritation test (ASIT), multi-irritation skin test (MIST), skin sensitization test (GPMT) and percutaneous penetration in vitro in experimental animals were performed respectively. Results: ASIT showed that none of the three chemicals was irritative. MIST revealed that cinnamic aldehyde was an irritant, and the others were not, the average index of skin irritation was consistent with the severity of histopathologic changes. GPMT showed all of the three chemicals were strong sensitizers. Percutaneous penetration of three kinds of perfumes in vitro showed all of them could be absorbed more or less, and the amount, rate and velocity of penetration of cinnamic aldehyde were the highest among them. Conclusion: The mechanism of cosmetic-related dermatitis may be due to primary irritation and/or allergy. The potency of skin sensitization of a chemical is related to its percutaneous penetration.

Gabiga H, Cal K, Kwiatkowski B, Janicki S, Brzoskowska-Paterska M. **[Comparative in vitro studies of percutaneous penetration of propylene glycol and oleic acid in the presence of some drugs in the formulation]**. Farm Pol 1997;53(5):195-9. (Pol)

Ganem-Quintanar A, Lafforgue C, Falson-Rieg F, Buri P. **Evaluation of the transepidermal**

permeation of diethylene glycol monoethyl ether and skin water loss. Int J Pharm 1997 Feb 28;147:165-71.
IPA COPYRIGHT: ASHP The transepidermal permeation of the absorption enhancer, ethoxydiglycol (diethylene glycol monoethyl ether; Transcutol), and epidermal water loss were studied using whole and stripped hairless rat skin in a modified vertical Franz diffusion cell. The presence of stratum corneum limited solvent penetration. However, after stripping of skin, the rate of ethoxydiglycol transfer increased and water flow rose until the vapor pressure at the skin surface and in ambient air was similar. The results suggested that ethoxydiglycol increased donor hydration by increasing the outflow of water, with a change in donor composition. It was noted that these 2 opposite fluxes and the alteration of the bulk constitution of skin and donor compartment throughout the study are important factors to be considered for permeation studies with this solvent.

Godwin DA, Michniak BB, Player MR, Sowell JW Sr. **Transdermal and dermal enhancing activity of pyrrolidinones in hairless mouse skin**. Int J Pharm 1997;155(2):241-50.
CBAC COPYRIGHT: CHEM ABS The transdermal penetration-enhancing abilities of 16 pyrrolidinones were tested against the model drug hydrocortisone using a hairless mouse skin model in vitro. Skins were pretreated for 1 h with the enhancer in propylene glycol before application of the drug also in propylene glycol. Enhancement ratios (ER) were calcd. for permeability coeff. (P), 24-h receptor concn. (Q24), and skin content of drug (SC) and compared to control values (no enhancer present). N-Dodecyl-2-pyrrolidinone and 2-pyrrolidinone-1-acetic acid dodecyl ester were found to produce the greatest enhancement for all the parameters calcd. with ER for Q24 of 23.11 and 11.68, resp. These same enhancers also produced high ERs for P with 42.95, resp. These values exceed Azone's enhancement ratios for the same parameters and hence these enhancers are candidates for further development as penetration enhancers.

Goffin V, Henry F, Pierard-Franchimont C, Pierard GE. **Topical retinol and the stratum corneum response to an environmental threat.** Skin Pharmacol 1997;10(2):85-9.
The functional consequences of using topical retinol on skin have not been thoroughly studied so far. The aim of this open study was to compare two preparations containing either retinol or vitamin E, using biometric evaluations. Three methods, namely the sodium lauryl sulfate (SLS) corneosurfametry bioassay, the ultraviolet (UV) squamometry test and optical profilometry of the UV-induced wrinkling process, were used to assess some properties of the stratum corneum. The retinol preparation achieved better scores than the vitamin-E cream in all three tests and appears to improve the resistance of the stratum corneum against some chemical (SLS) and physical (UV) threats. It also limits UV-induced shallow wrinkling.

Hashiguchi T, Ryu A, Itoyama T, Uchida K, Yamaguchi H. **Study of the effective dose of a topical antifungal agent, omoconazole nitrate, on the basis of percutaneous pharmacokinetics in guinea pigs and mice**. J Pharm Pharmacol 1997;49(8):757-61.
CBAC COPYRIGHT: CHEM ABS The clin. useful optimum dose of omoconazole nitrate, a topical antifungal agent, has been examd. by analyzing the percutaneous pharmacokinetics of the drug to assess its pharmacol. activity in an in-vivo study. Creams contg. omoconazole nitrate were prepd. on a pilot basis. The therapeutic effect of the omoconazole nitrate creams was examd. in an in-vivo pharmacol.

dermatophytosis infection model in guinea-pigs. Creams contg. 0.25% or higher concns. of omoconazole nitrate resulted in significant inhibition compared with no treatment and with vehicle-treated controls. In the mycol. examn. no growth of dermatophytes was obsd. for creams contg. 1% or higher concns. In an in-vitro hairless mouse skin-permeability test a non-linear least squares program based on a fast inverse Laplace transform algorithm was used to calc. the partition and diffusion parameters of omoconazole nitrate in the stratum corneum and viable epidermis. The time-course of drug concns. in the skin of the guinea-pig, estd. on the basis of these parameters, led to predictions that percutaneous drug concns. on the guinea-pig would require 10 or more days to reach equil. in the skin; that drug concns. in the corneum-viable epidermis border, where dermatophytes are considered to grow, would exceed the min. effective concn. when 0.1% or higher concn. creams were used; and that for binding to keratin drug concns. would reach the practical min. effective concn. when creams contg. 0.5% or more omoconazole nitrate were used. These results show that partition and diffusion parameters obtained from in-vitro skin permeation studies can be used to predict in-vivo percutaneous pharmacokinetics and to est. therapeutically effective concns.

Hostynek JJ, Magee PS. **Fragrance allergens: classification and ranking by QSAR.** Toxicol In Vitro 1997;11(4):377-84.
BIOSIS COPYRIGHT: BIOL ABS. Quantitative structure-activity relationship (QSAR) models which predict both skin penetration and cell mediated immunity for small molecular weight non-electrolytes developed earlier were validated on 74 known allergens and non-allergens chosen among fragrance chemicals in common use to test discriminating and grading power. While the test set used for classification was based on experience in humans exclusively, the rank model was tested for sensitization potency including guinea pig data also. In the classification test, 12 of 74 compounds fell in the indeterminate range and were non-classifiable by the present QSAR model. On the remaining 62 compounds the model performs with 90% sensitivity and 100% specificity at 92% concordance. The rank model correctly grades 65 of 74 compounds (88% concordance), with 60% specificity based on exact prediction of non-allergens (NON), and 95% sensitivity on allergens (ACD) allowing for a variance of : one level among weak, moderate and severe ratings. In combination, the two models perform with 93% overall concordance on the test set of 74 compounds.

Kasting GB, Francis WR, Bowman LA, Kinnett GO. **Percutaneous absorption of vanilloids: in vivo and in vitro studies**. J Pharm Sci 1997 Jan;86:142-6.
IPA COPYRIGHT: ASHP The percutaneous absorption of 3 radiolabeled vanilloids, nonivamide (vanillylnonanamide), olvanil (NE-19550), and NE-21610, was measured in vivo in the CD:VAF rat and in vitro through excised CD:VAF and SkH:Fz rat skin and human cadaver skin in various receptor solutions, and the dermal metabolism of the vanilloids was determined in vitro in SkH:Fz rat skin. The rank order of penetration in all species was nonivamide>olvanil>NE-21610. Rat skin was more permeable than human skin by factors ranging from 4 to 8 for nonivamide, 10 to 20 for olvanil, and about 10 to 100 for NE-21610. All 3 compounds were extensively metabolized in SkH:Fz rat skin. Of the various in vitro receptor solutions, only the preserved phosphate buffer containing a surfactant showed results that were in good agreement with those of the in vivo absorption studies for all 3 compounds up to 24 h post dose.

Kunta JR, Goskonda VR, Brotherton HO, Khan MA, Reddy IK. **Effect of menthol and related terpenes on the percutaneous absorption of propranolol across excised hairless mouse skin**. J Pharm Sci 1997;86(12):1369-73.
CBAC COPYRIGHT: CHEM ABS The potential use of terpenes/terpenoids as penetration enhancers in the transdermal delivery of propranolol-HCl (PL) was investigated. PL was chosen for the reasons of its extensive first-pass metab. and short elimination half-life. The terpenes studied included L-menthol, (+)-limonene, (.+-.)-linalool, and carvacrol at 1, 5, and 10% concns. The diffusion of PL across excised hairless mouse skin was detd. using side-by-side diffusion cells. Flux, permeability coeff. (Pm), and lag time (tL) were calcd. PL showed comparable lag times with menthol at all three concn. levels. At a 1% level of carvacrol, PL exhibited a 2.4- and 2.2-fold increase in lag time compared with 5 and 10% levels of enhancer, resp. In the presence of limonene, PL had shown max. lag time (between 3.0 and 3.3 h) at all 3 levels. In the case of linalool, the lag times for PL with 5 and 10% levels of enhancer were 7.0- and 5.2-fold less compared with 1% level. A significant concn. effect was obsd. only with linalool. Hydrogel-based patches were formulated with or without menthol as enhancer. Release profiles from the hydrogel formulations obeyed zero-order kinetics. The permeability of propranolol was significantly higher from the test patch than the control (no enhancer) patch across the mouse skin. The mechanism of permeation enhancement of menthol could involve its distribution preferenntially into the intercellula spaces of stratum corneum and the possible reversible disruption of the intercellular lipid domain. The results suggest the potential use of menthol as effective penetration enhancer in the delivery of significant amts. of PL through skin.

Lafforgue C, Carret L, Falson F, Reverdy ME, Freney J. **Percutaneous absorption of a chlorhexidine digluconate solution**. Int J Pharm 1997 Feb 28;147:243-6.
IPA COPYRIGHT: ASHP The percutaneous absorption of chlorhexidine gluconate (chlorhexidine digluconate; Hibitane) through hairless rat skin with or without stratum corneum was studied. For tests carried out on whole skin, storage in cutaneous structures after 48 h was more important than diffusion; the reverse was observed for stripped skin. When the skin was stripped, the amount absorbed was multiplied by approximately 100, and the amount stored in skin by approximately 10. The difference in chlorhexidine diffusion observed between whole and stripped skin was related to the physicochemical characteristics of chlorhexidine.

Lawrence JN. **Application of in vitro human skin models to dermal irritancy: a brief overview and future prospects**. Toxicol In Vitro 1997;11(3):305-12.
CBAC COPYRIGHT: CHEM ABS A review and discussion with many refs. The assessment of skin irritation potential represents an important component of the overall safety evaluation program for new and existing chems., which is predominantly performed in lab. animal studies. These in vivo skin irritancy tests have been subjected to much scientific condemnation with respect to their relevance to human risk assessment. The application of in vitro models in the initial assessment of skin irritation potential is regarded as desirable by the scientific community, but the development of such tests on a mechanistic basis is an important requirement with respect to validation of in vitro tests. The approach adopted in this paper is based on the hypothesis that a cellular insult and subsequent release of inflammatory mediators from the epidermal keratinocyte is a key initiating event in the development of an epidermal inflammatory response following exposure to an irritant. This reversible inflammatory

response is mechanistically distinct from a corrosive lesion resulting from the direct physicochem. interaction of a chem. with the stratum corneum. The potential of a chem. to elicit a corrosive response can be successfully predicted by in vitro systems using appropriate end points. The stratum corneum (dead cell layer) is also an important selective permeability barrier and a major factor in the severity of a subsequent biol. response following topical exposure, which must be taken into account in in vitro systems to assess less severe irritant responses. This paper emphasizes an in vitro mechanistic approach to human skin irritancy testing that is anticipated to facilitate safety assessment. The use of in vitro human skin models, incorporating aspects of barrier function and inflammatory mediator release, will contribute to an improved understanding of mechanisms underlying initiation of an epidermal inflammatory reaction and enable a crit. assessment of the value of this approach for human risk assessment purposes. It is not anticipated that in vitro human skin models per se will provide a definitive answer for dermal irritancy, but they appear promising as an initial assessment in the safety evaluation process.

Lawrence JN, Dickson FM, Benford DJ. **Skin irritant-induced cytotoxicity and prostaglandin E12 release in human skin keratinocyte cultures.** Toxicol In Vitro 1997;11(5):627-31.
BIOSIS COPYRIGHT: BIOL ABS. Damage to the skin induced by chemical irritants is associated with the release of arachidonic acid metabolites, such as prostaglandin E2 (PGE2) which plays an important role in epidermal inflammation. This study investigated cytotoxicity and release of PGE2 in human epidermal keratinocytes following an 18 hr exposure of confluent cultures to various skin irritants. The concentration-dependent release of PGE2 into the extracellular medium appeared to fall into two categories, which was reflective of possible mechanisms of action. Potent skin irritants, such as phorbol-12-myristate-13-acetate, benzalkonium chloride and tributyltin chloride, elicited an increase in extracellular PGE2 levels at concentrations that did not produce overt cell damage (uptake of neutral red at these concentrations was comparable to control levels). Non-irritants (2-methoxyethanol and 2-butoxyethyl acetate) and two less severe irritants (sodium dodecyl sulfate and acetic acid) stimulated release of PGE2-only at concentrations that compromised cellular integrity (uptake of neutral red was at least 50% lower than that of control cultures).

Leung H, Auletta CS. **Evaluation of skin sensitization and cross-reaction of nine alkyleneamines in the guinea pig maximization test**. J Toxicol Cutaneous Ocul Toxicol 1997;16(3):189-95.
CBAC COPYRIGHT: CHEM ABS A group of 9 alkyleneamines were investigated for their potential to induce skin sensitization and to cross-react with one another to elicit a hypersensitivity response. Ethylenediamine was the most potent skin sensitizer, and diethylenetriamine was next in potency. The sensitizing potency was inversely correlated with the no. of amine units. Cyclic amines such as piperazine had a lower sensitizing potency than the corresponding olefinic amines. Ethylenediamine also produced the strongest response in cross-reactions with other alkyleneamines. The results suggest that there was a direct correlation of the potencies to cause skin irritation, sensitization, and cross-sensitization in this family of alkyleneamines.

Liu KJ, Mader K, Shi X, Swartz HM. **Reduction of carcinogenic chromium(VI) on the skin of living rats.** Magn Reson Med 1997;38(4):524-6.
The reduction of chromate (Cr(VI)) on the skin of living rats was investigated using the technique of in

vivo electron paramagnetic resonance (EPR) spectroscopy with a surface coil resonator. Topical application of aqueous solution of Cr(VI) on the skin of rats generated the transient species, Cr(V). Partial removal of the stratum corneum increased the rates of formation and decay of Cr(V), as well as Cr (V) signal intensity. The results demonstrate that skin represents one route for chromium to enter into animals and humans. The generation of reactive Cr(V) species in the reduction of Cr(VI) by the skin may play an important role in the mechanism of Cr(VI)-induced skin cancer. These findings indicate the potential for in vivo EPR spectroscopy for studying the metabolism of paramagnetic reactive species in chemical and biochemical reactions occurring in/on the skin of both small and large animals, and possibly humans.

Loftsson T, Petersen DS, Le Goffic F, Olafsson JH. **Unsaturated glycerol monoethers as novel skin penetration enhancers**. Pharmazie 1997;52(6):463-5.
CBAC COPYRIGHT: CHEM ABS A mixt. of glycerol monoethers was extd. from the liver oil of deep sea shark (Centroporus squamosus). It consisted mainly of monoethers of glycerol and linear monounsatd. octadecanol, and glycerol and linear monounsatd. hexadecanol. Only about 11% of the ext. consisted of glycerol monoethers derived from linear satd. fatty alcs. The glycerol monoether ext. was somewhat less effective as skin penetration enhancer than oleic acid and other potent fatty acid penetration enhances, but it was still a very effective enhancer in the hairless mouse skin model used in this study.

Lopez A, Morant MJ, Guzman D, Borras-Blasco J, Herraez M, Et al. **Skin permeation model of phenylalkylcarboxylic homologous acids and their enhancer effect on percutaneous penetration of 5-fluorouracil**. Int J Pharm 1996 Aug 9;139:205-13.
IPA COPYRIGHT: ASHP To establish the rat skin penetration model of an acidic homologous series with a wide range of lipophilicity, the permeability and membrane/water partition coefficients of 6 phenylalkylcarboxylic acids were established and then underwent diffusion experiments then the effects of the acids on the penetration of fluorouracil (5-fluorouracil) were evaluated; effects of pH were also investigated. A linear relationship between the logarithms of permeabilities of penetrants and the corresponding membrane/water partition coefficients was found. Pretreatment with 1 of the acids increased the penetration of fluorouracil 4.5 times.

Mekenyan O, Roberts DW, Karcher W. **Molecular orbital parameters as predictors of skin sensitization potential of halo- and pseudohalobenzenes acting as SNAr electrophiles.** Chem Res Toxicol 1997;10(9):994-1000.
The electrophilic reactivity of a training set of 20 halo- and pseudohalobenzenes, 10 of which are reported skin sensitizers and 10 of which are reported nonsensitizers, has been modeled by MO-calculated indices using the AM1 and PM3 Hamiltonians. The electronic structures of parent molecules and the corresponding Meisenheimer intermediates (sigma-complexes) were evaluated. The NH2 group and the H atom were both studied as model nucleophile-derived substituents in the sigma-complexes. The LUMO energy differences between the parent compounds and their Meisenheimer complexes together with the maximum acceptor superdelocalizabilities determined over the aromatic reaction sites were found to discriminate correctly the sensitizing/reactive from nonsensitizing/unreactive compounds of the training set of 20 compounds. The predictive applicability of these MO indices was confirmed

with a test set of seven further compounds for which sensitization data are reported in the literature. A statistically based discriminant analysis provides a model which predicts whether or not an SNAr electrophile will be a sensitizer and estimates the degree of confidence in the prediction.

Monti D, Saettone MF. **Development of a transdermal patch for delivery of propafenone: preliminary studies in vitro**. STP Pharma Sci 1997;7(3):235-40.
IPA COPYRIGHT: ASHP Transdermal patch formulations containing propafenone base and hydrochloride were prepared using terpenes and laurocapram (Azone) as absorption enhancers, and drug penetration through hairless mouse skin was studied in vitro; patches containing propafenone hydrochloride and azone, the most favorable drug and enhancer combination, with and without a microporous membrane and adhesive layer, were evaluated using the same in vitro model. Skin, not membrane permeation, was the rate limiting step in delivery from the final patches. These patches, after 5-6 h lag times, delivered propafenone hydrochloride at a constant rate of 5.4-27.5 mcg/sq cm/h for more than 48 h. It was noted that the literature data for intravenous injections of propafenone indicate a need for higher rates.

Morreale M, Livrea MA. **Synergistic effect of glycolic acid on the antioxidant activity of alpha-tocopherol and melatonin in lipid bilayers and in human skin homogenates.** Biochem Mol Biol Int 1997;42(6):1093-102.
Considerable interest has been raised concerning the use of natural compounds in preventing skin aging and photoaging. In the idea that the combined action of agents increasing epidermal turnover with antioxidants could be advantageous in cosmetic and therapeutic treatments, we first investigated if alpha-glycolic acid affected or prevented the antioxidant activity of vitamin E and of melatonin, two compounds found beneficial as topical photoprotectant. Assays were carried out in vitro either in a biomimetic liposomal system, or in human skin homogenates. Lipid peroxidation was monitored spectrophotometrically by the time course of lipid hydroperoxide production in liposomes and by formation of TBA reactive substances (TBARS) in skin homogenates. Glycolic acid, at 25 microM to 1 mM, showed a mild, concentration-dependent antioxidant effect in liposomes, as evaluated by a slight decrease of the peroxidation rate, while, at 1 mM, reduced TBARS production in skin homogenates by 14%. Combinations of either vitamin E or melatonin with glycolic acid, in a 1:5 to 1:200 molar ratio, resulted in a clear synergistic protection of liposomes, more evident for the combination of glycolic acid with vitamin E. An amount of synergism up to 250% and up to 80% was evaluated with vitamin E and melatonin, respectively. Consumption rate of vitamin E during peroxidation of liposomes, in the absence or in the presence of glycolic acid, suggests that regeneration of vitamin E may in part explain the observed synergism. Synergistic antioxidant activity between vitamin E and glycolic acid was also observed in skin homogenates, whereas the effect of glycolic acid on the antioxidant activity of melatonin appeared additive. However, the combination of these three compounds inhibited TBARS production almost completely. Our data provide evidence that glycolic acid can strongly potentiate the antioxidant action of melatonin and vitamin E. This may suggest the advantage of combining alpha-glycolic acid with these antioxidants in skin designed preparations, both to improve penetration and availability of antioxidants to epidermal layers and to enhance their protective potential.

Noel-Hudson MS, Braut-Boucher F, Robert M, Aubery M, Wepierre J. **Comparison of six different**

methods to assess UVA cytotoxicity on reconstructed epidermis: relevance of a fluorimetric assay (the calcein-M) to evaluate the photoprotective effects of alpha-tocopherol. Toxicol In Vitro 1997;11 (5):645-51.
BIOSIS COPYRIGHT: BIOL ABS. A three-dimensional culture of human keratinocytes exposed at the air-liquid interface has been developed and used in conjunction with fluorimetric, colorimetric and radioligand incorporation assays to assess the in vitro toxicity of UVA. The aims of the study were: (1) to compare the relevance of the neutral red uptake (NR), MTT metabolism, 35S-methionine incorporation, IL1-alpha release and calcein-AM esterification assays for the evaluation of UVA injury; (2) to test the preventive protective effect of an emulsion containing 3% of tocopherol applied on the reconstructed epidermis, in comparison with an application of tocopherol 3% diluted in culture medium either on the apical compartment or in the underneath compartment of the skin culture insert. Viability measurement methods are based on different endpoints. None of the five endpoints measured produced LD50 values (40 J/cm2) that differed significantly from the others. However, calcein-AM assay was relatively more reproducible and easier to handle than the others, and seemed to be a better choice for the evaluation of the protective effects of the tocopherol emulsion. Tocopherol diluted in culture medium under the epidermis 24 hr before irradiation failed to protect the epidermis against UVA damage, whereas diluted in culture medium or in oily emulsion and applied to the epidermis reduced cellular death (cellular recovery values are, respectively, 24% and 21%). Since cosmetic or pharmaceutical formulations can be directly applied on the reconstructed epidermis as in vivo, this model in combination with a fluorescent viability assay appears to be a suitable approach for pharmaco-toxicological evaluations.

Ogiso T, Niinaka N, Iwaki M, Tanino T. **Mechanism for enhancement effect of lipid disperse system on percutaneous absorption. Part II.** Int J Pharm 1997;152(2):135-44.
CBAC COPYRIGHT: CHEM ABS To further clarify the mechanism involved in the enhancement effect of lipid disperse systems (LDS) on percutaneous absorption, the effect of particle size of LDSs on percutaneous absorption of betahistine (BH), the comparison of the enhancement effect of LDS with the lipid mixts. or the plain LDS, the effect of pretreatment of skin with gel formulation on penetration of LDS-BH and the fluidizing effect of LDSs on the stratum corneum (SC) lipids were estd. using Wistar and hairless rats. No major differences in BH absorption were seen between the gel formulations contg. LDS with three different particle size (128.+-.4, 336.+-.15, 596.+-.37 nm), prepd. using egg phosphatidylcholine (EPC), cholesterol and dicetylphosphate. The percutaneous absorbability of BH from the formulations contg. the lipid mixts. or plain LDS did not reach to the extent from EPC-LDS formulation. Following pretreatment with gel formulation contg. enhancer (D-limonene or n-octyl-beta-D-thioglucoside), BH absorption significantly decreased at the initial stage after application compared with that from LDS formulation, suggesting the additive enhancement effect of LDS and enhancer on the absorption. The treatment of the SC of hairless rat with LDSs significantly decreased the rotational correlation time (tauc) and shifted downwards the slope of curves (tauc vs. temp.) at temps. ranging from 25 to 60.degree.C, compared with that of untreated SC. However, the significant differences in the fluidizing effect between LDSs with different particle size were not obsd.

Okusa T, Obata Y, Takayama K, Higashiyama K, Nagai T. **Effect of menthol derivatives on skin permeation of oxybutynin**. Drug Delivery Syst 1997;12(5):327-33. (JPN)

CBAC COPYRIGHT: CHEM ABS Oxybutynin (OB) is widely used for the treatment of incontinence due to neurogenic bladder dysfunction. To reduce side effects and increase therapeutic efficiency, we investigated transdermal therapeutic system of OB. In vitro skin permeation study was performed by employing the vertical diffusion cell in which the excised rat abdominal skin was mounted, and menthol derivs. were selected as effective enhancers. The flux of OB was remarkably increased by the addn. of several kind of menthol derivs.(e.g. O-ethylether (MET), O-allyether, O-2-propynylether, O-methylester, O-propylester, O-isopropylester, and O-cyclopropylester). Among these compds., the skin irritancy of MET was significantly low compared with the other compds. Therefore, MET is thought to be a promising compd. as effective absorption enhancer for the transdermal drug delivery of OB. The data obtained from in vitro skin permeation study were analyzed by a membrane diffusion model derived from Fick's second law, and the diffusion and partition parameters of OB were estd. The diffusion parameters were increased when the menthol derivs. were incorporated in hydrogel. Partition parameters were almost const. The flux of OB increased with increase of the concn. of MET. Max. flux was obsd. when the hydrogel contg. 0.5% MET was applied. No further increase of flux was obtained when the amt. of MET was increased. In in vivo percutaneous absorption, the excretion of urine was restrained until 8 h by the administration of OB hydrogel contg. 0.5% MET. It was suggested that OB was delivered through the skin from hydrogel contg. 0.5% MET.

Riviere JE, Monteiro-Riviere NA, Inman AO. **The effect of altered media flow and glucose concentration on sulfur mustard toxicity in the isolated perfused porcine skin flap**. In Vitro Toxicol 1997;10(2):169-81.
CBAC COPYRIGHT: CHEM ABS The isolated perfused porcine skin flap (IPPSF), a novel alternative in vitro cutaneous model, has proven useful in the study of the pathogenesis of sulfur mustard (HD)-induced toxicity. The IPPSF is normally perfused at a media flow rate of 1.5 mL/min and glucose concn. of 120 mg/dL. This study assessed the effects of altered flow (0.5 or 3.0 mL/min) and glucose (50 or 400 mg/dL) on pathogenesis after ethanol control (EtOH) and HD (10.0 mg/mL) dosing. Pathogenesis in the flap was assessed by biochem. cumulative glucose utilization (CGU), physiol. (vascular resistance), and morphol. (light microscopy and TEM) end points. Vesication secondary to HD treatment was always assocd. with the formation of dark basal cells. The vascular resistance (VR) of HD-treated flaps increased while the CGU decreased with a decrease in media flow. High flow caused an increase in cytotoxicity in the control flaps, evidenced by severe intracellular epidermal edema, paranuclear vacuoles, and epidermal-dermal sepn. The VR was highest in the EtOH-treated low glucose flaps and the lowest in the HD-treated high glucose flaps. The CGU was directly proportional to the glucose concn. While low concns. of glucose resulted in blister formation and severe cellular cytotoxicity in the EtOH controls, high glucose blocked the formation of microvesicles and blisters in HD-treated IPPSFs despite moderate cytotoxicity. In summary, increased availability of glucose to the IPPSF modulates HD-induced cytotoxicity.

Roberts DW, Basketter DA. **Further evaluation of the quantitative structure-activity relationship for skin-sensitizing alkyl transfer agents.** Contact Dermatitis 1997;37(3):107-12.
The biological activity of skin-sensitizing chemicals can be expressed in terms of physicochemical properties which relate to the propensity of those chemicals to behave as electrophiles and which describe their ability to partition into the epidermis and between compartments within it. For defined

series of chemicals, it has proved possible to express such structure-activity relationships quantitatively. Such quantitative relationships can provide valuable insights into the mechanisms of skin sensitization and/or are of use in predictive toxicology. In the present work the quantitative structure-activity relationship (QSAR) previously derived for a series of alkyl transfer agents based on alkanesulfonate leaving groups has been critically examined in the light of skin sensitization data obtained for new members of that series and also for alkyl transfer agents based on different leaving groups. The QSAR predictions were broadly accurate, but demonstrated that further refinement was both necessary and possible. In particular, the physicochemical parameters which relate to the disposition of the chemical in the epidermis, i.e., its penetration through the stratum corneum, cell surface/cytoplasmic distribution and the associated dynamics, will need to be understood more fully in order to enhance the precision of the QSAR and its predictive power.

Rouget R, Schaeffer H. **Overview of in vitro cell culture technologies and pharmaco-toxicological applications.** Toxicol In Vitro 1997;11(5):591-9.
BIOSIS COPYRIGHT: BIOL ABS. Cutaneous-cell culture technologies have consisted initially of the isolation and maintenance of pure cutaneous cell types. Conventional (two-dimensional cultures) of keratinocytes or fibroblasts have yielded valuable information on the biochemistry and physiology of the epidermis or dermis. Subsequently, the aim of research has been co-culture of the various cell types to reconstitute in vitro the cellular interactions present in vivo. For example, co-culture of keratinocytes and melanocytes allow studies on mechanisms of melanogenesis. At the same time the reconstruction of skin as a tissue-like structure has allowed an improved differentiation of the epidermis and the development of dermatological applications. From a practical point of view, the three-dimensional culture of skin or epidermis is a decisive step in the development of in vitro cutaneous pharmaco-toxicology. Various models are now available with different advantages and limitations according to their composition and their handling. All of them allow the testing of water-insoluble materials including cosmetics and dermatological products, or of physical agents such as UV radiation. In the near future, the refinement of these models by introduction of other cell types such as melanocytes, Langerhans cells or endothelial cells, and improvement of the barrier function of the reconstructed stratum corneum, will permit their use in new fields in alternative methods.

Sartorelli P, Aprea C, Bussani R, Novelli MT, Orsi D, Sciarra G. **In vitro percutaneous penetration of methyl-parathion from a commercial formulation through the human skin.** Occup Environ Med 1997;54(7):524-5.
BIOSIS COPYRIGHT: BIOL ABS. Objective: To compare in vitro percutaneous absorption of methyl-parathion dissolved in an acetone vehicle and in the form of a commercial formulation. Methods: Penetration through the human skin was measured in Franz diffusion cells with full thickness skin from a human cadaver as the membrane. The two tailed non-parametric Mann-Whitney U test was used to compare the cumulative diffusion of methyl-parathion in the receptor fluid of the cells at various time intervals. Results: In vitro skin penetration of methyl-parathion was significantly higher with the commercial formulation. The percentage of the applied dose absorbed after 24 hours was 5.20% v 1.35%. The mean lag time was < 8 hours. Conclusion: Assessments of uptake and internal dose after exposure to pesticides should be based on the commercial products rather than active ingredients, because of the crucial role of the vehicle, as shown in this study.

Seko N, Bando H, Yamashita F, Takakura Y, Hashida M. **Estimation of drug concentration profiles in skin based on a skin diffusion model**. Drug Delivery Syst 1997;12(5):359-65.
CBAC COPYRIGHT: CHEM ABS This study was carried out to det. the possibility of estg. drug concn. profiles in skin via a diffusion model. In an in vitro permeation study using butylparaben as model drug, percent of drug permeated amounted to approx. 57% and 76% for intact and stripped skin resp., suggesting that the drug itself highly permeated through the stratum corneum. In vivo absorption study revealed that concn. of butylparaben decreased with skin depth where the max. was achieved at approx. 30 min and thereafter decreased gradually with time. To analyze both in vitro and in vivo percutaneous drug absorptions, Laplace transformed equations were derived based on four different kinds of hypothetical diffusion models. Initially, diffusion and partition parameters for butylparaben was calcd. by curve-fitting using nonlinear regression program combined with a fast Laplace transform algorithm (MULTI(FILT)). Using these parameters, further simulation studies of in vivo drug concn. profiles were carried out. When washout process due to blood flow was not taken into consideration, the decline of drug concn. profiles could hardly be stimulated. However, when model considering washout process to have occurred at the specific range in the dermis (50-200 mum from the stratum corneum/dermis boundary), this could fit the actual concn. profiles of butylparaben in skin well. Thus, this study demonstrated that the diffusion model enables us to evaluate the in vivo behavior of drug in skin.

Singh S, Khar RK. **Enhanced skin penetration of gentamicin sulfate by iontophoresis in vitro and in vivo studies**. Int J Pharm 1997;153(1):123-6.
CBAC COPYRIGHT: CHEM ABS In the present study enhanced permeation of gentamicin sulfate was achieved across rat skin by use of elec. current as compared to passive diffusion. A 65% increase in drug permeation was obtained in the in vitro release studies. In vivo studies showed a manifold increase in serum gentamicin sulfate concn. after iontophoresis.

Taguchi K, Takeuchi Y, Yamaoka Y, Fukushima S, Yasukawa H, Vasavada RC, Suzuki M. **Enhancement of propylene glycol permeation into rat dermis using high-purity oleic acid and subsequent alteration of the dermal protein structures**. Yakuzaigaku 1997;57(2):65-73.
CBAC COPYRIGHT: CHEM ABS The appearance of propylene glycol (PG) in rat dermis and subsequent protein structural alteration were examd. by measurement of PG spectra from the dermis and the spectra for amide regions from the dermal protein mols. Spectra were obtained using attenuated total reflectance IR spectroscopy following treatment of the rat skin with a high-purity oleic acid (HP-OA) / PG vehicle. HP-OA enhanced the PG flux in the dermis. The higher the concn. of HP-OA, the more quickly PG mols. distributed to the dermis and reached the maximal level (steady-state level). Detn. of the Amide II frequency in the dermis indicated that the structure of dermal proteins was altered by HP-OA, which implied that HP-OA entered into the dermis. The extent of such alteration was time-dependent and independent of HP-OA concn. The water content in the dermis did not change with time for at least 4 h after HP-OA treatment, indicating that the dermal tissue did not swell during the course of the expt. and that the spectral absorbance of water did not influence the spectra of amide II regions. The results of this study suggest that changes in the structure of dermal proteins must be considered as an important determinant for solute penetration through whole skin. In addn., detn. of the appearance of a solute, such as PG, in the dermis may be of value for characterizing the action of HP-OA, a skin

penetration enhancer.

Turowski M, Kaliszan R. **Keratin immobilized on silica as a new stationary phase for chromatographic modeling of skin permeation**. J Pharm Biomed Anal 1997;15(9-10):1325-33.
CBAC COPYRIGHT: CHEM ABS Skin permeability of org. compds. depends on their lipophilicity but can also be affected by compds. interactions with specific skin components. A good chromatog. model of percutaneous penetration detd. solely by lipophilicity is provided by the immobilized artificial membrane (IAM) columns. To complete the model a new high-performance liq. chromatog. (HPLC) stationary phase was prepd. by phys. immobilization of keratin on silica support. The keratin immobilized on silica has properties typical for the reversed-phase materials but it retains specifically acidic solutes. The keratin column can be used to conveniently compare keratolytic properties of xenobiotics. It was demonstrated that retention parameters detd. on a keratin column can be combined with the retention parameters detd. on the IAM column to predict differences in skin permeability within a class of drugs. It has been postulated that HPLC can model skin permeation thus reducing research time and costs as well as the use of lab. animals.

Walker M, Hulme TA, Rippon MG, Walmsley RS, Gunnigle S, Lewin M, Winsey S. **In vitro model(s) for the percutaneous delivery of active tissue repair agents**. J Pharm Sci 1997;86(12):1379-84.
CBAC COPYRIGHT: CHEM ABS There is a need to evaluate the permeability of human ulcerated tissue and periulcer tissue in order to assess the possible treatment of such a localized pathol. lesion with a topical therapy. In vitro percutaneous absorption studies were undertaken to evaluate an animal model that may mimic this clin. situation. Porcine skin from 3 anatomical sites, the ear, abdomen, and dorsum; ischemic skin (porcine and guinea pig); porcine wounds; and human skin (including periulcer and ulcerated tissue) were investigated, utilizing both whole skin and dermal membranes. Dermal membranes were chosen as representative of ulcerated tissue, as there would be no epidermal barrier present, and the thickness of the dermal membrane was not expected to offer any diffusional resistance to topically applied active agents. A range of chems. with differing physicochem. properties was investigated using a Franz type diffusion cell. For all tissues a permeability coeff. (kp with units of cm h-1) was measured, along with skin thickness and tissue partition coeff. measurements. Under these exptl. conditions and for the range of compds. tested, the results suggest that porcine skin, whole skin, and dermal membranes should be considered as good representative in vitro models for the topical delivery of compds. to human skin and ulcerated tissue, resp.

Weersink RA, Hayward JE, Diamond KR, Patterson MS. **Accuracy of noninvasive in vivo measurements of photosensitizer uptake based on a diffusion model of reflectance spectroscopy.** Photochem Photobiol 1997;66(3):326-35.
BIOSIS COPYRIGHT: BIOL ABS. This study compares the photosensitizer concentration measured noninvasively in vivo by diffuse reflectance spectroscopy with the results of postmortem tissue solubilization and fluorometric assay. The reflectance spectrometer consists of a fiber optic surface probe, spectrometer and charge-coupled device (CCD) array detector. The surface probe has eight detection fibers separated from the light source fiber by distances ranging from 0.85 to 10 mm. The imaging spectrometer disperses the light from each detector fiber onto the two-dimensional CCD array, while maintaining spatial separation of each individual spectrum. A single exposure of the CCD

therefore captures the reflectance spectrum at eight distances and over a range of 300 nm. From the spectra, the tissue's optical scattering and absorption coefficients are determined using a diffusion model of light propagation. Changes in the tissue absorption are used to estimate the photosensitizer concentration. Normal New Zealand White rabbits were injected with aluminum phthalocyanine tetrasulfonate (AlPcS4) and probe measurements made 24 h after injection on the dorsal skin, on muscle after surgically turning the skin back and on liver. For skin, the noninvasive estimate is proportional to the true concentration but low by a factor of 3. Based on Monte Carlo modeling of multilayered systems, this underestimate is attributed to the layered structure of the skin and nonuniform AlPCS4 distribution. A comparison of the noninvasive concentration estimates to the postmortem assay results finds good agreement for liver tissue even though application of the diffusion model is not strictly justified.

Yokomizo Y, Sagitani H. **Effects of phospholipids on the in vitro percutaneous penetration of prednisolone and analysis of mechanism by using attenuated total reflectance-Fourier transform infrared spectroscopy**. J Pharm Sci 1996 Nov;85:1220-6. IPA COPYRIGHT: ASHP The effects of 10 phospholipids on the in vitro percutaneous penetration of prednisolone through the dorsal skin of guinea pigs were investigated. A marked enhancing effect of prednisolone penetration was observed in the presence of phospholipids with unsaturated acyl chains. A maximum of 68-fold enhancement was observed compared to that of control. However, phospholipids with saturated acyl chains did not significantly increase the amount of prednisolone passing to the receptor side. Attenuated total reflectance-Fourier transform infrared spectroscopy was used to monitor the outer several microns of stratum corneum (SC) surface. Phospholipids with unsaturated acyl chains induced higher and broader absorbance shifts in the C-H bond stretching region while phospholipids with saturated acyl chains induced lower and sharper absorbance shifts in this region. A significant parallel between amount of prednisolone penetrated and lipid-chain fluidity of the SC was found. It was concluded that phospholipids may influence the percutaneous penetration of prednisolone by changing the lipid-chain fluidity of the SC.

Zhang Z, Monteiro-Riviere NA. **Comparison of integrins in human skin, pig skin, and perfused skin: an in vitro skin toxicology model**. J Appl Toxicol 1997;17(4):247-53.
CBAC COPYRIGHT: CHEM ABS To det. whether the isolated perfused porcine skin flap (IPPSF) could be utilized to study skin diseases mediated by integrins, the expression of integrins alpha2beta1, alpha3beta1, and alpha6beta4 was studied in human skin, pig skin, and the IPPSF using immunohistochem. staining. Immunostaining of both alpha2beta1 and alpha3beta1 was primarily located at the periphery of the basal keratinocytes in human skin. Similarly, alpha2beta1 was expressed in the stratum basale layer of the epidermis in both pig skin and the IPPSF after 8 h of perfusion. These antibodies defined the periphery of the pig basal keratinocytes more diffusely than that of human cells. However, the alpha3 antibody outlined the keratinocytes in all epidermal layers of the IPPSF and in the pig skin. In human skin, pig skin, and the IPPSF, alpha6beta4 stained exclusively at the basal pole of the basal keratinocytes, and showed a continuous linear labeling along the epidermal-dermal junction. The IPPSF showed stronger immunoreactivity with the antibody against beta4. Furthermore, the distribution of alpha6beta4 in 5.0 mg/mL of bis(2-chloroethyl) sulfide (sulfur mustard, HD)-induced blisters was examd. in the IPPSF. The alpha6beta4 staining was exclusively located on the epidermal side (roof) of the blister. In addn., alpha6beta4 staining was not linear but disrupted and patchy. These findings

suggest that any destruction of alpha6beta4 may weaken the epidermal-dermal junction, thereby leading to HD-induced vesication. This study demonstrates that the IPPSF expresses similar integrins to those of human skin, and the distribution of alpha6beta4 in the IPPSF blisters caused by HD is comparable to that of some human basement membrane blistering diseases. Therefore, the pig and the IPPSF prove to be ideal models to study the role of integrins in wound healing and blistering diseases occurring at the epidermal-dermal junction.

Zhong BZ, Whong WZ, Ong TM. **Detection of mineral-dust-induced DNA damage in two mammalian cell lines using the alkaline single cell gel/comet assay.** Mutat Res 1997;393(3):181-7. It has been estimated that over three million workers in the USA are potentially exposed to silica or other mineral dusts. Results of epidemiological studies evaluating whether silica or glass fibers increase lung cancer risk to the exposed workers are inconclusive. Detection of DNA damage in cells exposed to genotoxic agents is being used to assess the carcinogenic potential of environmental agents. The alkaline (pH > 13) single cell gel/comet (SCG) assay was used to determine and compare DNA damage in cultured Chinese hamster lung fibroblasts (V79 cells) and human embryonic lung fibroblasts (Hel 299 cells) exposed to crystalline silica (Min-U-Sil 5), amorphous silica (Spherisorb), carbon black, and glass fibers (AAA-10). V79 or Hel 299 cells were exposed to these mineral dusts for 3 h at various concentrations. Min-U-Sil 5 and AAA-10, at almost all concentrations tested, caused a significant increase in DNA migration measured as tail length in both V79 and Hel 299 exposed cells. However, the increase was much higher in V79 then in Hel 299 cells for Min-U-Sil 5. Tail length was also increased relative to controls after amorphous silica treatment, but not to the same extent as that induced by crystalline silica. Exposure to carbon black did not induce DNA migration at any of the concentrations tested. These results indicate that silica and glass fibers, but not carbon black, can induce DNA damage in mammalian cells, and that crystalline silica has a higher DNA-damaging activity than amorphous silica. For glass fibers, induction of DNA damage in both V79 and Hel 299 cells was observed even at a concentration 10 times lower than silica and the response was similar in both cell lines. These results suggest that the SCG/comet assay is useful for the detection of DNA damage caused by occupationally related dusts/particles.

ECOTOXCITY

Adam C, Garnier-Laplace J, Baudin JP. **Uptake from water, release and tissue distribution of 54Mn in the rainbow trout (Oncorhynchus mykiss Walbaum).** Environ Pollut 1997;97(1-2):29-38. BIOSIS COPYRIGHT: BIOL ABS. As part of a research programme on the transfer of several radionuclides along a pelagic trophic chain, two groups of 12 trout were kept for 8 weeks in water contaminated with 30 Bq ml-1 of 54Mn. In order to simulate chronic contamination and limit alterations in the physical and chemical characteristics of the medium, the water was renewed every 2 days. The kinetics of the accumulation and elimination of the radionuclide were monitored in one group of fish. The second group was used to study the contamination of the main organs and tissues at the end of the accumulation phase.The dynamics of contamination can be described by a bi-compartmental model, taking into account the fluctuations in the concentration of 54Mn in the water, as well as the biological dilution resulting from the growth of the fish. The theoretical value of the steady-state concentration factor for zero growth is 13 (w.w.) and the radionuclide release is characterised by two biological half-

lives of 6 and 97 days. At the end of the accumulation phase, the 54Mn is preferentially fixed in the bone, gills, skin and brain. The data obtained at the end of the depuration phase allow one to classify the organs in two groups with different elimination kinetics. The first group consists of organs of penetration or transit, such as the skin, gills, kidneys, liver, primary and secondary gut and viscera, whereas the second group is made up of the receptor and storage organs and tissues such as the bone, head, fins and muscle.

Berends AG, Boelhouwers EJ, Thus JL, De Gerlache J, De Rooij CG. **Bioaccumulation and lack of toxicity of octachlorodibenzofuran (OCDF) and octachlorodibenzo-p-dioxin (OCDD) to early-life stages of zebra fish (Brachydanio rerio).** Chemosphere 1997;35(4):853-65.
Previous studies with octachlorodibenzo-p-dioxin (OCDD) and octachlorodibenzofuran (OCDF) in juvenile or adult fish exposed via water revealed no toxicity, despite significant bioaccumulation. With 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), the fish early-life stage study has been shown to be the most sensitive test system. Therefore, the effects of OCDD and OCDF on the early-life stages of zebra fish (Brachydanio rerio) were determined during a flow-through test based on a column generator method. No statistically significant effect of OCDD and OCDF on the survival and hatching time of the eggs was found. Furthermore, no effects on survival, weight, general appearance or behaviour of the larvae were observed at the end of the exposure period of 32 days. GC-MS analysis of test solution samples revealed geometric mean measured concentrations of 32 (OCDD) and 34 ng/l (OCDF), respectively. Concentrations in surviving larvae at the end of the study were 61 (OCDD) and 94 (OCDF) micrograms/kg, respectively. These concentrations in zebra fish larvae were several orders of magnitude higher than concentrations in fish collected from the wild. In a review of the available laboratory fish experiments, we found a lack of biomagnification of OCDD and OCDF. We do not expect to find adverse effects of these compounds on the aquatic environment.

Boon JP, Van Der Meer J, Allchin CR, Law RJ, Klungsoyr J, Leonards PE, Spliid H, Storr-Hansen E, Mckenzie C, Wells DE. **Concentration-dependent changes of PCB patterns in fish-eating mammals: structural evidence for induction of cytochrome P450.** Arch Environ Contam Toxicol 1997;33(3):298-311.
Data sets on CB concentrations in fish-eating mammals from five laboratories were combined to test and refine a pharmacokinetic model. Clear differences in PCB patterns were observed between species. The ability to metabolize chlorobiphenyl (CB) congeners with vicinal H-atoms only in the ortho- and meta-positions and with one ortho-chlorine substituent generally increased in the order otter < cetaceans (harbor porpoise, common dolphin) < phocid seals (harbor and grey seal), but the metabolism of congeners with vicinal H-atoms in the meta- and para-positions and with two ortho-chlorines increased in the order cetaceans < seals < otter. Both categories of congeners are probably metabolized by different families of cytochrome P450 (1A and 2B) of which levels apparently differed between the cetaceans, the pinnipeds, and the otter. Within-species CB patterns differed in a concentration-dependent manner. The induction of cytochrome P450 enzymes offers the most likely explanation for this phenomenon, but starvation could have a similar effect on occasion.

Cope WG, Bartsch MR, Marking LL. **Efficacy of candidate chemicals for preventing attachment of zebra mussels (Dreissena polymorpha).** Environ Toxicol Chem 1997;16(9):1930-4.

BIOSIS COPYRIGHT: BIOL ABS. Forty-seven chemicals having potential for preventing the attachment of zebra mussels Dreissena polymorpha were identified and tested. For each chemical, 15 zebra mussels (5-8-mm shell length) in each of two replicates and six treatments were exposed for 48 h followed by a 48-h postexposure period in untreated water. Eleven of the chemicals inhibited the reattachment of zebra mussels after the 48-h exposure; eight had EC50 values ranging from 0.4 to 5.4 mg/L, and three had EC50 values ranging from 19.4 to 29.0 mg/L. Based on an analysis of chemical cost, solubility in water, anticipated treatment concentrations, and potential hazards to humans or the environment, three of the most promising chemicals, all antioxidants, (butylated hydroxyanisole (BHA), tert-butylhydroquinone, and tannic acid) were tested on nontarget fish (bluegill, Lepomis macrochirus; channel catfish, Icalurus punctatus; and rainbow trout, Oncorhynchus mykiss). These chemicals were not selectively toxic to zebra mussels; only the tests with bluegill and BHA and with channel catfish and tannic acid had 48-h LC50 values greater than the concentrations effective for preventing the reattachment of zebra mussels. Although the attachment of zebra mussels can be prevented with selected antioxidants, an alternative formulation should be investigated to minimize effects on nontarget organisms, such as fish.

De Coen W, Janssen CR. **The use of biomarkers in Daphnia magna toxicity testing. II. Digestive enzyme activity in Daphnia magna exposed to sublethal concentrations of cadmium, chromium and mercury.** Chemosphere 1997;35(5):1053-67.
BIOSIS COPYRIGHT: BIOL ABS. The effect of 48h and 96h exposure to sublethal concentrations of CdCl2, HgCl2 and K2Cr2O7 on the digestive enzyme activity of Daphnia magna was assessed. Five different digestive enzymes were investigated: cellulase, amylase, beta-galactosidase, trypsin and esterase. Both inhibition (CdCl2 and HgCl2) and increase (K2Cr2O7) of the enzyme activities were noted after short-term (48h) exposure. No inhibition, however, was observed after prolonged exposure (96h) to HgCl2 and K2Cr2O7 and even an increase in activity was noted for CdCl2. The increased digestive enzyme activity probably reflects the test organism's altered food assimilation efficiency in order to cope with the reduced food uptake. The observed changes in the digestive enzyme activity were furthermore evaluated as alternative effect criteria for chronic effect concentrations. Comparison of the enzymatic effect concentrations (1 and 2 enzyme LOEC's) with the LOEC's obtained for chronic reproduction tests (data taken from literature) shows that the concentration where at least 1 enzyme was significantly affected after only 48h exposure, was predictive of the 21 day chronic toxicity values.

Erbes M, Wessler A, Obst U, Wild A. **Detection of primary DNA damage in Chlamydomonas reinhardtii by means of modified microgel electrophoresis.** Environ Mol Mutagen 1997;30(4):448-58.
The assessment of genotoxic potential in surface water requires test methods, among which are those that detect initial DNA damage in organisms of aquatic biocenosis. The microgel electrophoresis (MGE) comet assay was applied to a ubiquitous unicellular green alga (Chlamydomonas reinhardtii) to detect DNA damage caused by genotoxins. For this, the test protocol described by Singh NP et al. [Exp Cell Res 175: 184-191, 1988] was modified. Major modifications were the use of alkaline lysis buffer with ionic detergents and the reduction of preincubation and electrophoresis times. Short-time exposure of Chlamydomonas to the well-known genotoxicants 4-nitroquinoline-1-oxide (4-NQO), N-nitrosodimethylamine, and hydrogen peroxide led to dose-dependent DNA damage. Chlamydomonas

responded very sensitively to treatment with increasing doses of 4-NQO. At a concentration of 25 nM, significant DNA damage was observed. At higher 4-NQO doses (> 100 nM), DNA damage was visible as complete DNA fragmentation into fine granules. N-Nitrosodimethylamine caused genotoxic effects at a concentration range from 0.014 to 0.14 mM without producing complete DNA fragmentation at the concentrations tested (highest dose, 140 mM). To evaluate the influence of illumination conditions during exposure, cells were incubated with increasing doses of H2O2 (0.25-1.0 mM) in darkness and in light. Our results indicate that incubation in light enables Chlamydomonas to cope with oxidative stress more efficiently than under dark conditions. To a certain extent, cytotoxic as well as genotoxic effects of H2O2 depend on the illumination condition or repair and anti-oxidative protection mechanisms activated by light, respectively.

Gagne F, Blaise C. **Evaluation of cell viability, mixed function oxidase activity, metallothionein induction, and genotoxicity in rainbow trout hepatocytes exposed to industrial effluents. II. Validation of the rainbow trout hepatocyte model for ecotoxicity testing of industrial wastewater.** Environ Toxicol Water Qual 1997;12(4):305-14.
BIOSIS COPYRIGHT: BIOL ABS. Primary cultures of rainbow trout hepatocyte are proposed as an alternative to the rainbow trout bioassay for evaluating mortality, hepatic metallothionein and cytochrome P4501A activity inductions, and hepatic DNA damage with industrial effluents. The validation study sought to assess the performance of the hepatocyte model (HM) in terms of specificity, predictive value, and concordance. In addition, correlation and artificial neural network analysis were used to model the cell system response compared with responses obtained in trout. The HM exhibited an overall sensitivity of 90% for detecting the various effects, suggesting that most of the time this method was able to detect effluents that were toxic to trout. The specificity ranged between 68 and 89%, indicating that hepatocytes were also able to confirm the absence of effects in effluent-exposed trout most of the time, but that in some cases the HM gave false positives, particularly when effects were measured at a concentration greater than 22%. The predictive values showed a similar range (i.e., 67-93%), suggesting that the HM was generally predictive of fish toxicity. The overall concordance ranged between 79 and 91%, indicating that responses obtained with the HM were consistent with the effects measured in effluent-exposed trout. The lower percentages obtained for specificity and predictive value can be explained by the fact that with some effluents the HM seemed to be more sensitive than the trout assay, since it displayed toxic effects even when none were detected in trout. In effluents that were concordant, a statistically significant linear regression model was derived so that trout toxicity/effect end points could be predicted from those obtained with the cell system. This validation study suggests that the rainbow trout hepatocyte model can be used as an alternative testing procedure to the rainbow trout assay. The cell system can be used as a prescreening tool to distinguish effluents that are likely toxic to fish from those that are not.

Gagne F, Blaise C. **Evaluation of industrial wastewater quality with a chemiluminescent peroxidation activity assay.** Environ Toxicol Water Qual 1997;12(4):315-20.
BIOSIS COPYRIGHT: BIOL ABS. The potential toxicity of industrial wastewater, or effluent, was evaluated with a very rapid and sensitive chemiluminescent peroxidase assay. The assay, based on the peroxidase-catalyzed oxidation of luminol by hydrogen peroxide, is responsive to the presence of radical scavengers and enzyme-inhibiting substances. The chemical competition of the chemicals between DNA

and the chemiluminescent peroxidase reaction was also investigated in order to evaluate the DNA-binding properties of these where DNA binding may lead to harmful adducts. The assay is rapid, requiring only 2-5 min incubation with the test sample at 21 C. For the DNA competition assay, DNA was added to the test sample 15 min before the incubation step in order to evaluate reversal of light inhibition or protection of luminescence. Of the 21 industrial effluents studied, 76% had an inhibiting effect on light emission, suggesting that these effluents are probably toxic. Indeed, it was found that these effluents were toxic with at least one of the following: the Microtox assay, the rainbow trout lethality test, and Ceriopdaphnia dubia survival/reproduction tests. It was found that this assay complied relatively well (60%) with the Microtox toxicity test, suggesting that the light inhibiting effects of the effluent correspond to toxic effects in bacteria. The addition of DNA to the reaction mixture prevented inhibition of light emission (i.e., DNA has a protective effect) in 29% of the effluents tested, suggesting that these effluents contained potentially genotoxic chemicals. Moreover, DNA-reactive effluents proved to be genotoxic to Escherichia coli (SOS Chromotest) most of the time (70%), suggesting that DNA competition is also related to DNA damage. No effluent displayed genotoxicity with the SOS Chromotest and did not show DNA binding with the chemiluminescent peroxidase assay. The chemiluminescent peroxidase assay may be used for prescreening studies when the number of test samples is very high or when information about the potential (geno)toxicity is urgently needed.

Gagne F, Blaise C. **Predicting the toxicity of complex mixtures using artificial neural networks.** Chemosphere 1997;35(6):1343-63.
BIOSIS COPYRIGHT: BIOL ABS. Industrial and municipal wastewaters constitute major sources of contamination of the aquatic compartment and represent a threat to aquatic life. Artificial neural networks based on three different learning paradigms were studied as a means of predicting acute toxicity to trout (5 days exposure to wastewaters) using input data from two simple microbiotests requiring only 5 or 15 min of incubation. These microbiotests were 1) the chemoluminescent peroxidase (Cl-Per) assay, which can detect radical scavengers and enzyme-inhibiting substances, and 2) the luminescent bacteria toxicity test (Microtox), in which reduction of light emission by bacteria during exposure is taken as a measure of toxicity. The responses obtained with the trout bioassay, the Cl-Per and the Microtox test were analyzed through statistical correlation (Pearson product-moment correlation), unsupervised learning by a self-organizing network, and assisted learning by the backpropagation and the Boltzmann machine (probabilistic) paradigms. No significant correlation ($p<0.05$) was found between the responses obtained with either the Cl-Per assay ($p = 0.121$) or the Microtox ($p = 0.061$) microbiotest and those resulting from the trout bioassay. The self-organizing network was able to identify by itself a maximum of five classes that were more or less relevant for predicting toxicity to fish: class 1 contained 2 samples that were toxic to fish, class 2 contained 2/3 samples that were toxic, class 3 showed 6/8 samples that were non toxic, class 4 contained 5/6 samples that were non-toxic and class 5 comprised one sample that was toxic. Supervised learning with backpropagation analysis yielded two kinds of networks that hold potential. The first one was able to predict the actual toxic wastewater concentration with an overall performance of 65% when fed fresh data, while the second one, which was designed to differentiate between toxic and non-toxic effluents, exhibited a much better performance (90%). However, the probabilistic network also proved to be a very good predictive model for toxicity to fish, with an overall performance of 90%. Although more data are needed, the network based on the backpropagation paradigm seems to be a better predictor or classifier

of trout toxicity when used with the Cl-Per and the Microtox microbiotests.

Huang XD, Krylov SN, Ren L, Mcconkey BJ, Dixon DG, Greenberg BM. **Mechanistic quantitative structure-activity relationship model for the photoinduced toxicity of polycyclic aromatic hydrocarbons: II. An empirical model for the toxicity of 16 polycyclic aromatic hydrocarbons to the duckweed Lemna gibba L. G-3.** Environ Toxicol Chem 1997;16(11):2296-303.
BIOSIS COPYRIGHT: BIOL ABS. Photoinduced toxicity of polycyclic aromatic hydrocarbons (PAHs) occurs via photosensitization reactions (e.g., generation of singlet-state oxygen) and by photomodification (photooxidation and/or photolysis) of the chemicals to more toxic species. The quantitative structure-activity relationship (QSAR) described in the companion paper predicted, in theory, that photosensitization and photomodification additively contribute to toxicity. To substantiate this QSAR modeling exercise it was necessary to show that toxicity can be described by empirically derived parameters. The toxicity of 16 PAHs to the duckweed Lemna gibba was measured as inhibition of leaf production in simulated solar radiation (a light source with a spectrum similar to that of sunlight). A predictive model for toxicity was generated based on the theoretical model developed in the companion paper. The photophysical descriptors required of each PAH for modeling were efficiency of photon absorbance, relative uptake, quantum yield for triplet-state formation, and the rate of photomodification. The photomodification rates of the PAHs showed a moderate correlation to toxicity, whereas a derived photosensitization factor (PSF; based on absorbance, triplet-state quantum yield, and uptake) for each PAH showed only a weak, complex correlation to toxicity. However, summing the rate of photomodification and the PSF resulted in a strong correlation to toxicity that had predictive value. When the PSF and a derived photomodification factor (PMF; based on the photomodification rate and toxicity of the photomodified PAHs) were summed, an excellent explanatory model of toxicity was produced, substantiating the additive contributions of the two factors.

Kajal N, Dhingra HR, Varghese TM. **Flowering and pollen germination of pea (Pisum sativum L.) genotypes raised in chromium-polluted soil**. Indian J Plant Physiol 1996;1(4):290-2.
CBAC COPYRIGHT: CHEM ABS The genotype PH 1 of pea (P. sativum L.) produced more flowers than HFP 4 under control condition. Chromium applied as potassium dichromate decreased flower prodn., the decrease being more pronounced in PH 1 than HFP 4. Pollen grains of both genotypes possessed >95% viability which remained unaffected by chromium treatment. Chromium did not influence in vitro germination potential of pollen but impaired tube growth of PH 1. Tube elongation in HFP 4 was nearly insensitive to test levels of chromium. Supplementation of chromium (upto 0.2 mM) to the germination medium did not affect germination but higher concns. inhibited it completely. Tube length decreased with increasing dosage of chromium and the decline was drastic at the 0.05-mM level. PH 1 was more sensitive to Cr6+ than HFP 4.

Meyn O, Zeeman M, Wise MJ, Keane SE. **Terrestrial wildlife risk assessment for TCDD in land-applied pulp and paper mill sludge.** Environ Toxicol Chem 1997;16(9):1789-801.
BIOSIS COPYRIGHT: BIOL ABS. A risk assessment was performed to evaluate the potential effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in land-applied pulp and paper mill sludge on terrestrial wildlife. Three representative bird and mammal species were assessed for potential individual risk of adverse effects. A dietary model was used to estimate TCDD exposures of adult birds and mammals,

and a pharmacokinetic model was used to estimate exposure for avian embryos. Using the quotient method, modeled exposure levels were compared to published no-observed-adverse-effect levels (NOAELs) for birds and mammals to calculate risk. Monte Carlo analysis was used to consider the variability and uncertainty in the risk estimates. The results suggest that TCDD in land applied pulp and paper sludge may pose significant individual risks to terrestrial wildlife under certain circumstances. Shrews were found to be most at risk due to their high consumption rate of food items that are expected to bioconcentrate the TCDD from soil at the application sites. Of all possible pathways, only dietary exposure was considered in this investigation. The analysis centered on parameter uncertainty and does not include an assessment of alternative models, although this could be a significant source of uncertainty.

Passino-Reader DR, Hickey JP, Ogilvie LM. **Toxicity to Daphnia pules and QSAR predictions for polycyclic hydrocarbons representative of Great Lakes contaminants.** Bull Environ Contam Toxicol 1997;59(5):834-40.
BIOSIS COPYRIGHT: BIOL ABS. RRM RESEARCH ARTICLE DAPHNIA-PULEX POLYCYCLIC HYDROCARBONS SITE SPECIFIC RISK ASSESSMENT TOXICOLOGY QSAR QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP ENVIRONMENTAL CONTAMINATION GREAT LAKES NORTH AMERICA.

Peijnenburg WJ, Posthuma L, Eijsackers HJ, Allen HE. **A conceptual framework for implementation of bioavailability of metals for environmental management purposes.** Ecotoxicol Environ Saf 1997;37(2):163-72.
Although bioavailability is an important issue, the scientific basis for its adequate use in the assessment of ecological risks is weak. What is often ignored is that bioavailability should be handled as a dynamic process that comprises two distinct phases: a physicochemically driven desorption process (also referred to as environmental availability) and a physiologically driven uptake process (also referred to as environmental bioavailability). Since the internal concentration of the organism (also referred to as toxicological bioavailability) is related with organ-effect levels, it is the latter that is determinant for the actual bioavailability. On the basis of contemporary ideas on equilibrium partitioning both within soils and between soils and organisms combined with a detailed literature review, in this contribution a framework is presented aimed at providing a guidance to necessary components of risk assessment procedures that take bioavailability into account. The framework provides suggestions with regard to the design and scope of studies to be carried out. It is based on knowledge on physico-chemical metal partitioning, in combination with models and concepts applied to analyse toxico-kinetics in exposed organisms. The conceptual dynamic framework boils down to a description of the system in the form of equilibria. It is assumed that each biotic species can be considered as one of the soil phases next to the particulate phase and the liquid phase. Each phase has a characteristic set of exposure routes. Equilibration processes are assumed to take place between all phases present. Essential is that the plan should result in validated procedures that, because they will explicitly address the issue of availability, will be predictive of effects in systems that have not been biologically tested.

Pratt JR, Melendez AE, Barreiro R, Bowers NJ. **Predicting the ecological effects of herbicides.** Ecol Applic 1997;7(4):1117-24.

BIOSIS COPYRIGHT: BIOL ABS. One purpose of the science of ecotoxicology is to provide information for protecting ecosystems. Understanding the hazards of chemicals is essential to wise decision making, and it is now clear that community structure changes are closely linked to altered ecosystem function. Uncertainty is high when decisions are made from a small biological (toxicological) database. Individual bioassays provide little insight into biological interactions that are important in sustaining ecosystems. Artificial ecosystem experiments with herbicides demonstrate the limited predictive power of bioassays and ecological risk assessment methods that are heavily dependent on animal testing. Many herbicides interfere with unique pathways in photosynthetic organisms but are not very toxic to animals. For example, the herbicide atrazine is not considered toxic to fishes, because atrazine interferes with electron transport in photosystem II. But, adding atrazine at low levels (3-100 mug/L) to aquatic microcosms demonstrated significant increases in algal biomass, concurrent enhancement of nutrient recovery systems, and increases in the detectable number of heterotrophic microbial species. Higher levels of atrazine (100-300 mug/L) produced general collapse of these laboratory ecosystems. Low levels of atrazine capable of producing ecosystem-level effects can occur from days to weeks in streams of midwestern agricultural areas. Conversely, the herbicide diquat is rapidly immobilized in the field if fine sediments are present. Laboratory bioassays tend to overestimate diquat toxicity if sediments are not present because the material rarely persists in the water column. A variety of measures of ecosystem condition are available for assessment of chemical effects. Community structure changes (especially of nontarget groups) and changes in ecosystem process variables have technical importance and are not assessed in current risk assessment paradigms. Regulators need to draw on a more comprehensive data set than is presently used to make risk assessment decisions. Sometimes, this may require using methods other than those considered standard for data development.

Rao SS, Neheli T, Carey JH, Cairns VW. **Fish hepatic micronuclei as an indication of exposure to genotoxic environmental contaminants.** Environ Toxicol Water Qual 1997;12(3):217-22.
BIOSIS COPYRIGHT: BIOL ABS. An in vivo assay protocol for detecting hepatic micronuclei in fish was performed to evaluate whether this genotoxic response could serve as a bioindicator of environmental exposure to genotoxic substances. The incidence of hepatic micronuclei was compared in brown bullheads (Ameiurus nebulosus) with external lesions collected from a contaminated site and fish showing no externally visible pathologies collected from reference sites. Laboratory experiments were performed by exposing hatchery-raised rainbow trout (Onchorhynchus mykiss) to a pulp mill effluent extract (XAD-4-NaOH) with known genotoxic activity. Both bullhead and trout were injected with allyl formate to induce hepatic necrosis and regenerative proliferation of hepatocytes prior to examining the liver tissue for micronuclei. An elevated incidence of hepatic micronuclei was observed in Hamilton Harbour bullheads showing visible lesions relative to the micronucleus incidence in bullheads from reference sites with no external pathologies. Similarly, rainbow trout exposed to pulp mill effluent extracts exhibited an elevated incidence of hepatic micronuclei compared to controls. These results suggest that the hepatic micronucleus assay could serve as a valuable biomonitoring tool for assessing the impacts of genotoxic environmental contaminants on fish.

Rimkus GG, Butte W, Geyer HJ. **Critical considerations on the analysis and bioaccumulation of musk xylene and other synthetic nitro musks in fish.** Chemosphere 1997;35(7):1497-507.
BIOSIS COPYRIGHT: BIOL ABS. Several analytical methods for the residue analysis of musk xylene

(MX), musk ketone (MK), and other nitro musks in fish are presented and discussed. In particular because of a publication about a fast and simple analysis without any clean-up procedure, the necessity of a thorough clean-up of fat-containing samples is emphasized. In addition, the present knowledge about the high bioconcentration of these lipophilic pollutants in fish is summarized. In a long-term bioconcentration study we found in rainbow trouts bioconcentration factors (BCF) on a wet weight basis (BCFw) of MX from 4,200 to 5,100 while the BCF values on a lipid basis (BCFL) ranged from 115,000 to 122,000. These BCF values of MX are in good agreement with the BCF values calculated from the noctanol/water partition coefficient (log Kow = 4.90) using the Quantitative Structure-Activity Relationship (QSAR). A published study with extremely low bioconcentration factors of MX is critically discussed.

Sauer TC, Costa HJ, Brown JS, Ward TJ. **Toxicity identification evaluations of produced-water effluents.** Environ Toxicol Chem 1997;16(10):2020-8.
BIOSIS COPYRIGHT: BIOL ABS. Toxicity identification evaluations (TIEs) were performed on 14 produced-water (PW) samples of various salinities from inland and offshore oil- and gas-production facilities operated by different companies in Wyoming, Texas, California, and Louisiana (USA) to evaluate the efficacy of TIE procedures in determining potential toxicants in PW effluents. The research involved acute (24- and 48-h) freshwater and marine toxicity tests on whole PW and PW fractions generated by standard U.S. Environmental Protection Agency and PW-specific fractionation schemes. Factors influencing PW TIEs were investigated, such as the effect of salinity in selecting fractionation manipulations, the effect of toxicity test replication (i.e., reproducibility) in distinguishing changes in toxicities between whole PW and its fractions, and the suitability of different test species in PW TIEs. The results obtained and lessons learned from conducting these PW TIEs are presented in this article. Components, or fractions, contributing to toxicity differed for each PW with no specific fraction being consistently toxic. For most PW samples, toxicity attributed to any one fraction represented only part of the toxicity of the whole sample. However, no more than two fraction types were identified as potential toxicants in any sample. Potential toxicants identified during this study, besides salinity, included acidic and basic organic compound class fractions, particulates removed by filtration at pH 11, ammonia, hydrocarbons, hydrogen sulfide, material removed by pH change, and volatile compounds.

Sauvant MP, Pepin D, Bohatier J, Groliere CA, Guillot J. **Toxicity assessment of 16 inorganic environmental pollutants by six bioassays.** Ecotoxicol Environ Saf 1997;37(2):131-40.
The relative toxicity of 16 environmental pollutants, such as inorganic elements (Ba, Cd, Co, Cr, Cu, Fe, Ge, Hg, Mn, Nb, Pb, Sb, Sn, Ti, V, and Zn), is evaluated on the L-929 established cell line of murine fibroblasts, with five bioassays [RNA synthesis rate assay (RNA), MTT reduction assay (MTT), neutral red incorporation assay (NRI), Coomassie blue assay, and cellular growth rate assay], and on the ciliated protozoa Tetrahymena pyriformis GL [doubling time of T. pyriformis GL population assay (DTP)]. For each inorganic substance, the six bioassays allowed the toxicological index IC50 (inhibitory concentration 50%) to be calculated. The IC50 values are useful to rank the tested elements and to compare the features of the six bioassays. The most sensitive assays were the RNA, MTT, NRI, and DTP assays. Moreover, the in vitro IC50 values correlated with the in vivo LD50 values; these results were close to those obtained with established lines of human, murine, or fish cells. The sensitivity and the complementarity of these bioassays would be in favor of their incorporation in a battery of tests used

for toxicological screening studies of xenobiotics.

Sherry J, Scott B, Dutka B. **Use of various acute, sublethal and early life-stage tests to evaluate the toxicity of refinery effluents.** Environ Toxicol Chem 1997;16(11):2249-57.
BIOSIS COPYRIGHT: BIOL ABS. The toxicities of effluents from three Ontario, Canada, refineries were assessed with microbes, plants, invertebrates, and fish. Acute toxicity was assessed by the Microtox test, an assay based on electron transport activity in submitochondrial particles, and Daphnia magna (water flea) and Oncorhynchus mykiss (rainbow trout, 96-h exposure) bioassays. Short-term toxic effects were measured with bioassays of growth and survival of Pimephales promelas (fathead minnow) larvae; survival and reproduction of Ceriodaphnia dubia (water flea); growth of Selenastrum capricornutum (alga); growth of Lemna minor (aquatic plant); germination of Lactuca sativa (nonaquatic plant); survival, growth, and maturation of Panagrellus redivivus (nematode); and genotoxicity in the SOS-Chromotest. Only the Microtox test and the submitochondrial particle test detected acute toxicity in the effluent samples. Reduced survival and sublethal responses were caused by some effluents, but not all effluents were toxic, and none caused a response in all of the tests applied. The results suggest that the effluent treatment systems used at Ontario refineries have largely eliminated acute toxicity to the organisms in our test battery. Although reduced survival and sublethal effects were detected in some of the effluents, the effects were minor. Some of the tests provided evidence, albeit weak, of variations in the responses of the test organisms to a temporal series of effluent samples. Not unexpectedly, there were also minor differences in the responses of the tests to effluents from the three refineries. The fathead minnow test seems to be a sensitive indicator of the sublethal toxicity of Ontario refinery effluents.

St-Louis R, Pelletier E, Marsot P. **A mechanistic approach to tributyltin (TBT) sorption by marine microflagellated alga Pavlova lutheri.** Appl Organometal Chem 1997;11(7):543-50.
BIOSIS COPYRIGHT: BIOL ABS. The sorption of tributyltin (TBT) by phytoplankton cells was studied by exposing the marine microflagellated alga Pavlova lutheri grown in batch culture to TBT chloride concentrations ranging from 0.37 to 74 nm (0.1 to 21 mug l-1) for a 24-hour period. The phytotoxicity of TBT was indicated by a decrease in cell density at all TBT concentrations used. TBT was tightly bound to the surface cell and was only washed out by a strong acidic solution (pH:3) while inner cell-absorbed TBT was only recovered by means of an organic solvent. The sorption of TBT by P. lutheri occurred via two mechanisms: a passive ionic surface adsorption followed by a facilitated intracellular absorption. The coupling/uncoupling of these two mechanisms was dependent upon the contamination level used. The cell surface adsorption of TBT was best described by the Freundlich adsorption model whereas the obstruction of the facilitated absorption mechanism was apparently related to the toxic activity of TBT towards cell components.

Thomann RV, Shkreli F, Harrison S. **A pharmacokinetic model of cadmium in rainbow trout.** Environ Toxicol Chem 1997;16(11):2268-74.
BIOSIS COPYRIGHT: BIOL ABS. It has been previously observed that exposure of rainbow trout to Cd from water or food did not result in a steady state for key compartments such as the gill, liver, and kidney. Further, during deputation, the mass of Cd in the kidney continued to increase. A seven-compartment pharmacokinetic model of the disposition of cadmium in the rainbow trout was constructed

to obtain insight into these observations. The model considers exchange across the gill from exposure to dissolved (available) Cd in the water and exchange of Cd across the gut wall due to exposure to Cd in the food source. Internal distribution of Cd is via Cd in blood exchanging with aqueous phase Cd in kidney. liver, and a storage compartment. Equilibrium partitioning is assumed between the aqueous phase Cd and bound tissue Cd in each compartment. The model is applied to a data set where trout were exposed under two conditions: Cd primarily in water and primarily in food. The model parameters were obtained from other published exposure experiments as well as calibration to the data. The parameters were not altered between the two exposure pathways. High surface gill sorption and gut biliary transfer were necessary in order to obtain reasonable model calibration. Reproduction of the observed increase in kidney Cd during deputation is obtained with a relatively high partitioning, and model flux calculations indicate a net flux into the kidney during the deputation phase. Model simulations for both water and food exposure routes indicated that the whole body Cd concentration was calculated to reach equilibrium in about 50 d. However, Cd did not achieve a steady state in the kidney where it reached a maximum concentration at seven times whole body. For assessment of Cd risk to trout target tissues, it is concluded that a pharmacokinetic model may be necessary.

Urrestarazu Ramos E, Vaes WH, Verhaar HJ, Hermens JL. **QSARs in predictive environmental toxicology. Polar narcosis. Designing a suitable training set for QSAR studies**. Environ Sci Pollut Res Int 1997;4(2):83-90.
CBAC COPYRIGHT: CHEM ABS For 97 polar narcotics, which are not ionized under physiol. conditions, 11 physico-chem. and quantum-chem. descriptors were calcd. Using principal component anal., 91% of the total variance in this descriptor space were explained by 3 principal components which were subsequently used as factors in a statistical design. Eleven compds. were selected based on a 2-level full factorial design including 3 compds. near the center of the chem. domain (a 23+3 design). Quant. structure-activity relationships (QSARs) were developed for both the design set and the whole set of 63 polar narcotics for which guppy and/or fathead minnow data were available in the literature. Both QSARs, based on partial least squares regression (3 latent variables), resulted in good models and provided similar pseudo-regression coeffs. The model based on the design chems. was able to predict the toxicity of the 63 compds. Models show that acute fish toxicity is detd. by hydrophobicity, HOMO-LUMO energy gap, and H-bond acceptor capacity.

Vaes W Hj, Ramos EU, Hamwijk C, Van Holsteijn I, Blaauboer BJ, Seinen W, Verhaar H J, Hermens J L. **Solid phase microextraction as a tool to determine membrane/water partition coefficients and bioavailable concentrations in in vitro systems.** Chem Res Toxicol 1997;10(10):1067-72.
BIOSIS COPYRIGHT: BIOL ABS. Solid phase microextraction (SPME) is an extraction technique that uses a polymer-coated fiber as the extraction device. After extraction, the compound of interest can be desorbed from the fiber and subsequently analyzed by GC or HPLC. One of the properties of SPME is that only the freely dissolved fraction of a chemical is available for partitioning to the extraction device. The method can be applied in a way that small amounts are extracted from the sample, which allows negligible depletion extraction. These two properties make SPME devices particularly suitable for measurements of free concentrations. In toxicological studies the free concentration is considered to be a more relevant parameter, concerning toxic effects, than the nominal concentration that is used most frequently. In the current study, the usefulness of this method to measure phospholipid/water partition

coefficients and free concentrations in three different in vitro test systems (rat hepatocytes in primary culture, 9000g and 100000g homogenate fractions of rainbow trout liver) was demonstrated. Results show separate relationships between phospholipid/water and n-octanol/water partition coefficients for a set of polar and nonpolar organic chemicals, respectively. These observations suggest that phospholipid/ water partition coefficients may be a more suitable parameter in modeling the kinetic behavior of organic chemicals. Additionally, differences between the nominal and the actual free concentration in in vitro systems are more pronounced for more hydrophobic compounds, as was expected based on theoretical considerations. To our knowledge, the approach presented here is the first analytical method to measure toxicologically relevant concentrations in in vitro test systems in a fast and efficient way.

Wang G, Bai N. **Study on QSAR for general pollutants in organic industrial waste**. Toxic Subst Mech 1997;16(4):315-26. CBAC COPYRIGHT: CHEM ABS The quant. structure-activity relationships between acute toxicity of 88 general pollutants, such as phenols, alcs., alkanes, and alkenes, in org. industrial waste to Daphnia magna (24-h IC50) and 8 mol. descriptors are studied by an expert system approach. It shows that the correct classification rate reaches 95% in a training set including 68 compds., and the correct prediction rate is about 90% in a predicting set including 20 compds. Some rules used in the expert system are interpreted by calcg. quantum chem. parameters, Etumo and Ehomo.

White PA, Blaise C, Rasmussen JB. **Detection of genotoxic substances in bivalve molluscs from the Saguenay Fjord (Canada), using the SOS chromotest.** Mutat Res 1997;392(3):277-300. BIOSIS COPYRIGHT: BIOL ABS. Few studies have employed bioassays to investigate the accumulation of genotoxins in aquatic biota that inhabit areas contaminated with industrial and municipal wastes. This study employed the SOS Chromotest, a short-term bacterial genotoxicity assay, to investigate the presence of genotoxins in bivalve molluscs from the Saguenay Fjord (Canada). Genotoxicity analyses were performed on dichloromethane extracts of Mya arenaria and Mytilus edulis collected downstream from several aluminum refineries and forestry products industries known to produce and release genotoxic substances. The results confirmed that bivalve molluscs inhabiting downstream regions are contaminated with both direct-acting and pro-genotoxic substances. In several cases, SOS response induction factors exceeded 3.0. The results failed to reveal a clear downstream trend of decreasing genotoxicity with increasing distance from the presumed industrial sources(s). A significant relationship ($r2 = 0.61$, $p < 0.007$) between a demographic variable (population near shoreline) and lipid-corrected genotoxic potency suggest that the accumulated direct-acting genotoxins may be of municipal origin. Significant relationships between tissue extract genotoxicity ($r2 = 0.75$, $p < 0.003$) and tissue PAH contamination ($r2 = 0.77$, $p < 0.0001$) and drainage basin area suggests that the bivalves are accumulating airborne contaminants deposited on the surface of the relevant drainage basins. In spite of contamination with genotoxic PAHs, the addition of rat liver microsomal enzymes reduced the genotoxic potency of all samples investigated (31-94% decrease). The results also revealed a significant relationship between tissue extract genotoxicity and PAH concentration ($r2 = 0.72$, $p < 0.0005$). Further analyses confirmed that a variable portion (7-97%) of the S9-activated tissue extract genotoxicity can be attributed to the detected PAHs. Although the sources, identity and effects of genotoxins accumulated by bivalves of the Saguenay Fjord remain to be determined, the study has confirmed the utility of the SOS Chromotest in environmental monitoring of aquatic biota.

Wong D C, Dorn PB, Chai EY. **Acute toxicity and structure-activity relationships of nine alcohol ethoxylate surfactants to fathead minnow and Daphnia magna.** Environ Toxicol Chem 1997;16 (9):1970-6.
BIOSIS COPYRIGHT: BIOL ABS. Aquatic toxicity of nine commercial-grade alcohol ethoxylate surfactants was studied in acute exposures to fathead minnow (Pimephales promelas) and Daphnia magna. All studies were conducted in accordance with USEPA TSCA Good Laboratory Practice Standards. Mean measured surfactant concentrations in the exposure solutions showed good agreement with nominal concentrations for both fathead minnow (R2 = 0.98, p = 0.05) and daphnid (R2 = 0.99, p=0.05) tests. Surfactant recoveries ranged from 59 to 97% and 67 to 106% in the fathead minnow and daphnid solutions, respectively. Response of both species to the surfactants was generally similar. Surfactant toxicity tended to increase with increasing alkyl chain lengths. The effect of low average ethylene oxide (EO) groups on increased surfactant toxicity was more evident in the daphnid exposures. Quantitative structure-activity relationship (QSAR) models were developed from the data, which relate surfactant structure to toxicity. The models predict increasing toxicity with decreasing EO number and increasing alkyl chain length. The models also indicate that average alkyl chain length has a greater effect on toxicity than average EO groups. Further, the models indicate that both species did not differ markedly in their response to alkyl chain length effects, while the number of EO groups had a stronger effect on daphnids than fathead minnow. Model-predicted toxicity agreed well with toxicity estimated from USEPA alkyl ethoxylates SARs and with toxicity values reported in the literature for several surfactants previously studied.

GENOTOXICITY AND MUTAGENESIS

7th International Conference on Environmental Mutagens. Toulouse, France, 7-12 September 1997. Abstracts. Mutat Res 1997;379(1 Suppl 1):1-220.

Abramsson-Zetterberg L, Grawe J, Zetterberg G. **Spontaneous and radiation-induced micronuclei in erythrocytes from four species of wild rodents: a comparison with CBA mice.** Mutat Res 1997;393 (1-2):55-71.
Almost 100 animals of 4 different species of small wild rodents (bank vole, Clethrionomys glareolus; field vole, Microtus agrestis; yellow-necked mouse, Apodemus flavicollis; and wood mouse, Apodemus sylvaticus) were trapped in central Sweden and used in experiments to determine the spontaneous and radiation-induced frequencies of polychromatic (fMPCE) and normochromatic erythrocytes (fMNCE) from bone marrow (bm) and peripheral blood (pb) using flow cytometric analysis. The results were compared with those from similar experiments with CBA mice. The saving of time and labour by the use of the flow cytometer-based analysis was a prerequisite for this study in which about 135 million PCE were analysed. The two species of voles had a mean background fMPCE (bm) of about the same value as CBA mice, while the yellow-necked mice had about five times higher fMPCE (bm). Wood mice had more than twice the fMPCE (bm) compared to CBA mice. Between individual animals in each of the 4 species, the background fMPCE (bm) varied more than between individual CBA mice, and the elimination of micronucleated erythrocytes was considerable. When exposed to ionizing radiation, the voles did not show a significant response. The response of the two Apodemus species was similar to that of the CBA mice, although it varied between individual animals and was not correlated to their

background fMPCE. This study indicates that bank voles and field voles are unsuitable testing objects in the in vivo micronucleus assay. On the other hand, yellow-necked mice and wood mice seem to be useful in this test. Since the variation between individuals is considerable in wild Apodemus mice, large groups will be needed for obtaining statistically significant results when exposure to a genotoxic agent is low. Alternatively, repeated samples can be taken from individual wild mice to study the effect of a decreased exposure after keeping the animals for a period of time in an uncontaminated environment.

Abril N, Luque-Romero FL, Prieto-Alamo MJ, Rafferty JA, Margison GP, Pueyo C. **Bacterial and mammalian DNA alkyltransferases sensitize Escherichia coli to the lethal and mutagenic effects of dibromoalkanes.** Carcinogenesis 1997;18(10):1883-8.
Here we confirm and extend our previous studies demonstrating that the mutagenic potency of 1,2-dibromoethane (DBE) and dibromomethane (DBM) is markedly enhanced (not prevented) in bacteria expressing the O6-alkylguanine-DNA alkyltransferase (ATase) encoded by the Escherichia coli ogt gene. We demonstrate that, in close parallel with mutagenesis, the Ogt ATase sensitizes the bacteria to the lethal effects of these carcinogens, suggesting that one or more of the potentially mutagenic lesions induced by DBE and DBM in the presence of Ogt has additional lethal capacity. We further demonstrate that the sensitization to both lethality and mutagenesis by DBE and DBM is a property shared by other DNA alkyltransferases. This objective was accomplished by quantifying the induction of mutations and lethal events in ogt- ada- E. coli expressing an exogenous bacterial or mammalian ATase from a multicopy plasmid. Mammalian recombinant ATases enhanced the lethal and mutagenic actions of DBE and suppressed the lack of sensitivity of the vector-transformed bacteria to DBM. In most cases the order of effectiveness of the ATases ranked: murine > human > Ogt > rat. Further comparisons included the full-length Ada ATase from E. coli and a truncated Ada version (T-ada) that retains the O6-methylguanine binding domain of the protein. The full-length Ada ATase was effective in enhancing the lethality but not the mutagenicity induced by DBE and DBM. The T-ada ATase provided less sensitization than Ada to lethality by DBE, but of the three bacterial ATases T-ada yielded the highest sensitization to mutagenesis by this compound. T-ada and Ada ATases were in general less effective than the mammalian versions, with the exception of the rat recombinant ATase. The effectiveness of the different mammalian and bacterial ATases in promoting the deleterious actions of dibromoalkanes was compared with the effectiveness of these proteins in suppressing the lethal and mutagenic effects induced by N-nitroso-N-methylurea. The ability to sensitize E. coli to the lethal and mutagenic effects of DBE and DBM seems restricted to DNA alkyltransferase, since overexpression of thioredoxin (Trx) or glutaredoxin (Grx1) in ogt- ada- cells showed no effect, in spite of the reported potential of bioactive dihaloethane-derived species to alkylate Trx.

Anderson D, Basaran N, Dobrzynska MM, Basaran AA, Yu TW. **Modulating effects of flavonoids on food mutagens in human blood and sperm samples in the comet assay.** Teratog Carcinog Mutagen 1997;17(2):45-58.
The flavonoids silymarin, myricetin, quercetin, kaempferol, rutin, and kaempferol-3-rutinoside have been examined in combination with the food mutagens 3-amino-1-methyl-5H-pyrido (4,3-b)indole (Trp) and 2-amino-3-methylimidazo-4,5-f)quinoline (IQ) in the Comet assay in human lymphocytes from donors A and B and human sperm from donor B. These compounds alone have been shown to produce positive responses in the Comet assay, as have the food mutagens. However, in combination with the

food mutagens, the flavonoids produced antigenotoxic effects since DNA damage was reduced in the Comet assay in human lymphocytes and sperm over a similar dose range in the absence of metabolic activation. Only quercetin and kaempferol were examined in blood with metabolic activation, but there was no difference in response to that obtained without activation. In the blood there was an exacerbation or synergy of response at the lowest doses of the flavonoids. In the sperm this was also the case with silymarin and myricetin. With kaempferol there was no antigenotoxic effect and quercetin protected below baseline levels. Since the effects were observed in lymphocytes and sperm over a similar dose range, it would suggest that the Comet assay responses occur in somatic and germ cells in a one-to-one ratio. These results have implications for man in terms of risk assessment and in the modulation of isolated food constituents.

Anderson D, Dobrzynska MM, Yu TW. **Modulating effects of silymarin and myricetin on food mutagens and doxorubicin in assays with different genetic endpoints.** J Environ Pathol Toxicol Oncol 1997;16(4):313-27.
BIOSIS COPYRIGHT: BIOL ABS. Silymarin and myricetin, a flavonolignan and flavonoid respectively, have been used in combination with the food mutagens, 3-amino-1-methyl-5H-pyrido (4,3-b)indole (Trp), 2-amino-1-methyl-6-phenyl-imadazo(4,5-b)pyridine (PhIP), and 2-amino-3-methylimidazo-(4,5-f) quinoline (IQ) in the Ames test and Comet assay in human lymphocytes from donors A and B. Silymarin and myricetin alone have been shown to produce positive responses. Silymarin has also been investigated in combination with the anti-cancer drug doxorubicin in the chromosome aberration assay in donor B. Antigenotoxic effects have been observed since reversion was reduced with the food mutagens Trp and IQ in combination with silymarin and myricetin at the highest doses in the Ames test. DNA damage was also reduced in the Comet assay in combination with the food mutagens in both donors in the absence of exogenous metabolic activation, but not in its presence with the food mutagen PhIP in both donors. There was also an exacerbation or synergy of response at the lowest doses of silymarin and myricetin on some occasions. In the chromosome aberration assay, silymarin itself produced chromosome damage and a slight exacerbation or synergy in combination with doxorubicin. These results may have implications for humans in terms of modulating the genotoxic effects of isolated food constituents or drugs.

Ankathil R, Kusumakumary P, Nair MK. **Increased levels of mutagen-induced chromosome breakage in Down syndrome children with malignancy.** Cancer Genet Cytogenet 1997;99(2):126-8.
Even though an association between Down syndrome (DS) and malignancies has been established, the mechanism behind this is still unclear. We therefore investigated constitutional chromosomal abnormalities and bleomycin-induced chromosome sensitivity in 12 DS children, 8 DS children with malignancies, and 10 normal controls to explore whether these factors play any role in cancer predisposition. Trisomy 21 was the only constitutional cytogenetic abnormality observed in all the DS children. But there was significant variation between the patients and controls with regard to bleomycin sensitivity. Compared to the normal controls, all the DS patients expressed significantly higher chromosomal breaks per cell (b/c) values indicating sensitivity to bleomycin. Furthermore, DS children with malignancies demonstrated significantly higher b/c values than DS children with malignancies. These results permit us to assume that DS children showing mutagen hypersensitivity may be having defective DNA repair competence and hence may be predisposed to malignancies.

Auer H, Oehler R, Lindner R, Kowalski H, Sliutz G, Orel L, Kucera E, Simon MM, Glossl J. **Characterisation of genotoxic properties of 2',2'-difluorodeoxycytidine.** Mutat Res 1997;393(1-2):165-73.
The genotoxic properties of 2',2'-difluorodeoxycytidine (dFdC) were characterised using diploid, mortal low-passage fibroblasts (LPF cells) and the spontaneously transformed fibroblast cell line V79. In both cell types, incorporation of dFdC into the DNA led to an increase of DNA single-strand breaks evaluated by an in situ nick translation assay and to an accumulation of cells in the S-phase of the cell cycle. At concentrations below those leading to cell cycle arrest, dFdC neither induced sister chromatid exchange (SCE) nor structural chromosome aberrations in LPF cells, whereas V79 cells accumulated SCEs as well as chromosome breaks over a broad dose range. In LPF cells treated with dFdC, chromosomal alterations were detected by the micronucleus assay within a narrow concentration range, whereas in V79 cells, a dose-dependent increase in the appearance of micronuclei was seen up to cytotoxic concentrations. In addition, V79 cells went into apoptosis, as evaluated by nuclear fragmentation and condensation, whereas this phenomenon was not detectable in LPF cells.

Ballering LA, Nivard MJ, Vogel EW. **Preferential formation of deletions following in vivo exposure of postmeiotic Drosophila germ cells to the DNA etheno-adduct-forming carcinogen vinyl carbamate.** Environ Mol Mutagen 1997;30(3):321-9.
DNA sequence changes induced in the vermilion gene of Drosophila following in vivo treatment of postmeiotic male germ cells with vinyl carbamate (VCA), an etheno-adduct-forming carcinogen, are primarily deletions. With VCA, 65% (13/20) of the vermilion mutants isolated from crosses of NER+ (nucleotide excision repair) males with NER+ females and 40% (6/15) obtained from matings with NER- females were intra- or multi-locus deletions. Due to the insufficiently low mutagenic activity in NER+ genotypes of vinyl bromide (VB), another epsilon-adduct-forming carcinogen, vermilion mutants could only be isolated from crosses of VB-treated males with NER- females. Of 14 vermilion mutants induced by VB, three carried large deletions. Twenty-two of 23 base substitutions derived from either VCA or VB experiments fell into one of the four categories expected from epsilon-adducts: three vermilion mutants had GC-->AT transitions, five had AT-->GC transitions, 7 carried GC-->TA transversions, and 7 were AT-->TA transversions. In view of the similarities in the response of mouse and Drosophila germ lines to several classes of alkylating agents, a high incidence of deletions is predicted to occur as well in postmeiotic germ cells of mice exposed to these types of agents.

Barentsova ER, Khromykh YU. **[Interaction of mutations of the mei-9, mei-41, and rad201 genes for mutagen sensitivity in Drosophila exposed to ionizing radiation]**. Genetika 1997;33(3):328-32. (Rus)
BIOSIS COPYRIGHT: BIOL ABS. 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 and physical factors, while rad201 mutants are sensitive only to ionizing radiation. The results obtained showed that the interaction of mei-9 and rad201 mutations is additive and the interaction of mei-41 and rad201 mutations is epistatic. the maternal effect was demonstrated to be characteristic of all mutants tested even when larvae were exposed to radiation at late stage of development.

Bethke B, Sauer B. **Segmental genomic replacement by Cre-mediated recombination: genotoxic stress activation of the p53 promoter in single-copy transformants.** Nucleic Acids Res 1997;25 (14):2828-34.
Genotoxic stress results in transcriptional activation of the p53 promoter. To gain more detailed information on genotoxic induction of the p53 promoter at a uniform genomic locus, we have developed an efficient strategy for replacing a defined genomic segment in mouse NIH 3T3 cells with exogenous transfected DNA using a 'double lox' targeting strategy mediated by Cre DNA recombinase. The strategy utilizes a pair of heterospecific lox sites engineered both into the genome and onto the targeting DNA. This allows direct replacement of genomic DNA by a Cre-catalyzed double crossover event. p53-CAT reporter constructs were site-specifically placed into the genomic target 20-fold more efficiently by double lox recombination than by Cre-mediated single crossover insertional recombination, and the absolute frequency of site-specific double lox targeting exceeded the frequency of transformation due to random illegitimate recombination of transfected DNA into the genome. Resulting targeted single-copy integrants of the p53-CAT reporter show strong genotoxic induction by mitomycin C, and a dynamic range of induction that exceeds that seen in transient transfection assays. The double lox strategy is generally applicable to Cre-mediated genomic targeting in any cell and should be of particular utility in the site-specific targeting of DNA into embryonic stem (ES) cells for the production of gene-modified mice.

Bogadi-Sare A, Brumen V, Turk R, Karacic V, Zavalic M. **Genotoxic effects in workers exposed to benzene: with special reference to exposure biomarkers and confounding factors.** Ind Health 1997;35(3):367-73.
The aim of the study was to establish a correlation between biomarkers of exposure and cytogenetic test results in workers occupationally exposed to benzene and toluene, with special reference to confounding factors influencing the outcome of the cytogenetic test. The incidence of structural chromosome aberrations and sister chromatid exchanges was studied in the peripheral blood lymphocytes cell genome of 49 female shoe-makers, mean age 38 years, mean length of occupational exposure 17 years and in a group of 27 well-matched controls. Workers were exposed to concentrations of benzene up to 15 ppm and of toluene up to 50 ppm. The presence of benzene and toluene in the workers' blood samples, and the presence of phenol in pre- and post-shift urine were considered proof of occupational exposure. Chromosomal aberration analysis revealed a significant increase in dicentric incidence in the exposed group compared to the controls ($P = 0.004$). However, significant correlation between cytogenetic test results and the exposure biomarkers was not established. On the contrary, correlation between the cytogenetic test results and data on confounding factors (e.g. age and alcohol consumption), was marked. The major point raised by this study is the influence of confounding factors on the cytogenetic test outcome. This imposes the need for caution in the interpretation of cytogenetic test results, and ultimately in the estimation of individual genotoxicity risk related to low level benzene exposure.

Bombick DW, Bombick BR, Ayres PH, Putnam K, Avalos J, Borgerding MF, Doolittle DJ. **Evaluation of the genotoxic and cytotoxic potential of mainstream whole smoke and smoke condensate from a cigarette containing a novel carbon filter.** Fundam Appl Toxicol 1997;39(1):11-7.
A novel carbon filter has been developed which primarily reduces the amount of certain vapor phase constituents of tobacco smoke with greater efficiency than the charcoal filters of cigarettes currently in

the market. In vitro indicators of genotoxic and cytotoxic potential were used to compare the cigarette smoke condensate (particulate phase) or whole cigarette smoke (vapor phase and particulate phase) from cigarettes containing the novel carbon filter with smoke condensate or whole smoke from commercial or prototype cigarettes not containing the novel carbon filter. Ames bacterial mutagenicity, sister chromatid exchange (SCE) in Chinese hamster ovary (CHO) cells, and neutral red cytotoxicity assays in CHO cells were utilized to assess the genotoxic and cytotoxic potential of the cigarette smoke condensates. SCE and neutral red cytotoxicity assays were utilized to assess the genotoxic and cytotoxic potential of the whole smoke. As expected, the novel carbon filter did not significantly affect the genotoxic or cytotoxic activity of the smoke condensate, although we did observe that the use of low-nitrogen tobacco reduced the mutagenicity of the condensate in Salmonella typhimurium strain TA98. However, the whole smoke from cigarettes containing the novel carbon filter demonstrated significant reductions in genotoxic and cytotoxic potential compared to cigarettes without the novel carbon filter. The toxicity of the smoke was correlated ($r = 0.7662$ for cytotoxicity and $r = 0.7562$ for SCE induction) to the aggregate mass of several vapor phase components (acetone, acetaldehyde, acrolein, acrylonitrile, 1,3-butadiene, ammonia, NOx, HCN, benzene, isoprene, and formaldehyde) in the smoke of the cigarettes utilized in this study. In conclusion, this novel carbon filter, which significantly reduced the amount of carbonyls and other volatiles in mainstream cigarette smoke, resulted in significant reductions in the genotoxic and cytotoxic activity of the smoke as measured by these assays.

Boreiko AV, Krasavin EA. **[Mutagenic action of radiation with different LET on Bacillus subtilis cells]**. Radiats Biol Radioecol 1997;37(3):408-12. (Rus)
 The induction of the his- - his+ mutants in vegetative and spores of Bacillus subtilis wild type cells irradiated with gamma-rays and helium ions (LET = 20-80 keV/mum) has been investigated. It was shown that the dose dependence of the mutation induction in vegetative cells is described by a linear-quadratic function of dose in case of both gamma-rays and helium ions. RBE (LET) dependencies on the lethal and mutagenic effect of irradiation have a local maximum. The maximum of RBE (LET) dependence on the mutagenic assay is shifted at the low region of LET in comparison with the lethal effect of irradiation.

Brendler-Schwaab SY, Herbold BA. **A new method for the enrichment of single renal proximal tubular cells and their first use in the comet assay.** Mutat Res 1997;393(1-2):175-8.
A protocol was developed to isolate and enrich single renal proximal tubular cells, performing the following steps: in situ kidney perfusion; isolation of renal tissue pieces by collagenase digestion; selective enrichment of proximal tubular fragments by Percoll gradient centrifugation; and isolation of single proximal tubular cells by digestion of proximal tubular fragments with trypsin. The mean enrichment rate, determined by the glucose-6-phosphatase staining method, was 78.9% with a mean cell viability of 93.8%. After modification of the comet assay protocol, genotoxicity in proximal tubular cells could be investigated. A dose-dependent genotoxic effect of ethyl methanesulphonate in these cells was proven.

Chae S, Kafer E. **Two uvs genes of Aspergillus nidulans with different functions in error-prone repair: uvsI, active in mutation-specific reversion, and uvsC, a recA homolog, required for all UV mutagenesis**. Mol Gen Genet 1997;254(6):643-53. Two genes of

Aspergillus nidulans are known to function in UV mutagenesis, but have been assigned to different epistasis group: uvsC, which is also required for meiosis and mitotic recombination, and uvsI, which may have no other function. To clarify their role in error-prone repair and to investigate their interaction, uvsI and uvsC single and uvsI:uvsC double mutant strains were further tested for mutagen sensitivities and characterized for effects on mutation. Spontaneous and induced frequencies were compared in forward and reverse mutation assays. All results confirmed that uvsI and uvsC are members of different epistasis groups, and demonstrated that these uvs mutants have very different defects in UV mutagenesis. The uvsI strains showed wild-type frequencies in all forward mutation tests, but greatly reduced spontaneous and UV-induced reversion of some, but not other, point mutations. In contrast, uvsC had similar effects in all assay systems: namely pronounced mutator effects and greatly reduced UV mutagenesis. Interestingly, the uvsI:uvsC double mutant trains differed from both single mutants; they clearly showed synergism for all types of reversion tested: none were ever obtained spontaneously, nor after induction by UV or EMS (ethylmethane sulfonate). Based on these results, the authors conclude that uvsI is active in a mutation-specific, specialized error-prone repair process in Aspergillus. In contrast, uvsC, which is now known to show sequence homol. to recA, has a basic function in mutagenic UV repair in addn. to recombinational repair, similar to recA of Escherichia coli.

Charlotte Olsen L, Aasland R, Fjose A. **A vasa-like gene in zebrafish identifies putative primordial germ cells**. Mech Dev 1997;66(1-2):95-105.
CBAC COPYRIGHT: CHEM ABS The vasa gene is essential for germline formation in Drosophila. Vasa-related genes have been isolated from several organisms including nematode, frog and mammals. In order to gain insight into the early events in vertebrate germline development, zebrafish was chosen as a model. Two zebrafish vasa-related genes were isolated, p110a and vlg. The p110a gene was shown to be widely expressed during embryogenesis. The vlg gene and vasa belong to the same subfamily of RNA helicase encoding genes. Putative maternal vlg transcripts were detected shortly after fertilization and from the blastula stage onwards, expression was restricted to migratory cells most likely to be primordial germ cells.

Cimino MC, Auletta AE, Dearfield KL. **Response to selecting chemicals and assays for assessing mammalian germ cell mutagenicity. M. D. Shelby. Mutation Research 1996;352:159-67 and reply**. Mutat Res 1997;3799(1):105-6.
BIOSIS COPYRIGHT: BIOL ABS. RRM LETTER MAMMAL MAMMALIAN GERM CELL MUTAGENICITY EPA NATIONAL TOXICOLOGY PROGRAM MUTAGENS MUTATION TOXICOLOGY GENETIC TOXICITY MUTAGENICITY TESTING SCHEME GOVERNMENT AGENCY.

Cole J, Beare DM, Waugh A P, Capulas E, Aldridge KE, Arlett CF, Green M H, Crum JE, Cox D, Garner RC, et al. **Biomonitoring of possible human exposure to environmental genotoxic chemicals: lessons from a study following the wreck of the oil tanker Braer.** Environ Mol Mutagen 1997;30 (2):97-111.
BIOSIS COPYRIGHT: BIOL ABS. In January 1993 the oil tanker Braer ran aground in the Shetland Islands, Scotland. Approximately 80,000 tons of crude oil were released. Exceptionally high winds caused extensive pollution and exposure of the local population to crude oil. We describe the study

which was immediately set in place to examine the exposed population for evidence of genotoxic exposure. Blood samples were taken and primary DNA damage was measured in the mononuclear cell fraction by the butanol modification of the 32P-postlabelling method. Mutation was measured at the hprt locus in T lymphocytes. No evidence of genotoxicity was obtained for either end point, but nevertheless, we believe that useful lessons were learnt, which should be incorporated into the design of future studies: (1) A rapid response is essential, and even if sufficient funds are not immediately available, it is still worth attempting to obtain samples quickly and use cryopreservation, also to attempt to estimate exposure. (2) Adequate numbers of volunteers must be sought, together with enough controls, not just to allow meaningful analysis but to overcome loss of samples and failure of things to go according to plan. (3) Points concerning laboratory practice include: (i) samples should be coded, (ii) clearly defined and proven protocols should be used, (iii) irreplaceable samples should not be used for method development, (iv) should a problem become apparent during the study, work on such samples should cease immediately until the problem is solved, (v) all critical experimental components should be pretested against a laboratory standard. (4) The study design should include replicate experiments to monitor experimental variability and reproducibility, as well as internal standards and cryopreserved in house samples. Care must be taken that samples from any one exposure group are spread between a number of independent experiments and that each experiment includes samples from a number of exposure groups. (5) A computerised data base should be maintained with full details of experimental variables, donor attributes, and raw data so that any contribution of experimental artefacts to outlier results can be monitored. (6) Because of the nature of the statistical variation for many environmental genotoxicity end points, only a large-scale study is likely to be capable of yielding useful information.

Combes RD. **Statistical analysis of dose-response data from in vitro assays: an illustration using Salmonella mutagenicity data.** Toxicol In Vitro 1997;11(5):683-7.
BIOSIS COPYRIGHT: BIOL ABS. One of the advantages of in vitro methods is ease of testing multiple concentrations of chemicals for dose responses. A well defined response is used to confirm toxicity, especially for marginal effects, and a significant dose response alone indicates some effect of treatment for further clarification. For sublethal processes (e.g. enzyme induction, organelle damage or mutagenesis), decreases in response can arise at high dose levels that kill cells (cytolethality). This compromises statistical analysis of dose responses using standard approaches, such as ranking, which do not allow for omission of cytolethal dose data. An alternative is the recursive, non-parametric SM (1/2)/ Jonckheere-Terpstra test, where omission of results is permitted. Use of this approach is illustrated in conjunction with recommended statistical analyses (Dunnett's 't'-test and Wahrendorf ranking) for non-parametric data from Salmonella mutagenicity assays where the problem is frequently encountered. It is shown that the recursive test can be used for analysing non-parametric dose responses from in vitro assays, where decreases in response are seen at high test chemical concentrations.

Dahouk S, Plappert U, Gerngross H, Willy C. **[Systemic genotoxic effect caused by ischemia-/ reperfusion-/surgical trauma of the lower extremity--detection of DNA chain breaks in leukocytes with the Comet Assay]**. Langenbecks Arch Chir Suppl Kongressbd 1997;114:601-5. (Ger)
Ischemia-reperfusion-injury (IRI) represents a fundamental common pathway of tissue damage in a wide variety of disease processes, i.e. myocardial infarction, septic or hemorrhagic shock, multiple organ failure, trauma and organ transplantation. IRI is said to be initiated by leukocyte accumulation and

adhesion to vascular endothelium as well as oxygen free radicals (OFR) playing a pivotal role in the pathogenesis of ischemia-reperfusion-injury. However, only few data exist for measuring influence of toxic OFR on DNA in humans. To assess the potential genotoxicity the single-cell gel electrophoresis (COMET assay) of white blood cells was used in BTB-operated humans before and after 0, 5, 15, 30 and 120 min of tourniquet-ischemia of the lower limb (n = 20). We show that tourniquet-ischemia (60-170 min) significantly increased the tailmoment measuring the DNA-damage in the operated limb to 173.4% (median in % baseline (bl); Q0.35: 149.9%; Q0.75: 214.5%; $p < 0.01$ vs bl) and in the systemic circulation to 157.8% (Median in % bl; Q0.25: 136.8%; Q0.75: 174.8%; $p < 0.01$ vs bl). These results indicate that the IRI induces a local and systemic breaks indicating an overwhelming of the antioxidative barrier of circulating human leucocytes.

Dass SB, Heflich RH, Casciano DA. **The mutagenic response at the ouabain resistance locus in T cells of mice exposed to N-ethyl-N-nitrosourea parallels the response at the Hprt locus and correlates with mutation target size.** Carcinogenesis 1997;18(11):2233-7.
The lymphocyte Hprt gene has been used extensively as a reporter locus to monitor the mutational effects of the exposure of animals to genotoxicants. Implicit in this view of the function of a reporter gene is the assumption that its mutagenic response is representative of that of other genes in the organism. As a test of this hypothesis we compared the frequency of 6-thioguanine-resistant (TGr) mutants at the Hprt locus with the mutant frequency (MF) induced at another locus, the ouabain resistance (Oua) locus. The frequency of spontaneous OUA(R) mutants was estimated to be 1.1x10(-7) (MF between <0.3 and 1.1x10(-7)), which was approximately 30-fold less than the spontaneous TGr MF. Following treatment with N-ethyl-N-nitrosourea (ENU), the induced OUA(R) MF at each of two dose levels (50 and 150 mg/kg ENU) and two time points (3 and 6 weeks post-exposure) was consistently 8- to 9-fold lower than the corresponding TGr MF. Thus the mutagenic response of the Oua locus closely paralleled that of the Hprt locus, indicating a similarity in their response to ENU. In addition, the Oua locus was 3-4 times more sensitive than the Hprt locus to the mutagenic effect of ENU, as measured by the fold increase in MF over the background level. The number of ENU-mutable sites capable of resulting in a TGr or OUA(R) phenotype, otherwise known as the mutation target size, was estimated to differ by an order of magnitude between the two loci. This difference in target size correlates with, and therefore may largely account for, the difference in induced MF between both loci.

Deng XS, Tuo J, Poulsen HE, Loft S. **2-Nitropropane-induced DNA damage in rat bone marrow.** Mutat Res 1997;391(3):165-9.
DNA damage detected by the comet assay (single cell gel electrophoresis) and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) formation in DNA in the bone marrow has been studied in groups of 6 male Wistar rats treated with a single i.p. injection of the carcinogen 2-nitropropane (2-NP, 100 mg/kg body weight) or vehicle. Twenty-four hours after 2-NP the average tail length in the comet assay in bone marrow cells was increased from 1.46 +/- 0.27 to 9.61 +/- 1.56 microm (mean /- SD, $p < 0.01$), and 8-oxodG levels in the DNA were increased from 1.04 +/- 0.50 to 5.14 +/- 2.42 per 10(5) dG ($p < 0.01$). There was a close correlation between the comet tail length and the 8-oxodG level ($r = 0.89$, $p < 0.05$). The results indicate that 2-NP inflicts DNA damage in the bone marrow cells and thus could be leukemogenic.

Dobrovol'skaya MA, Spiegelman VS, Belitskii GA. **Newly constructed insecticide-resistant D. melanogaster strains with elevated sensitivity to the mutagenic effect of benzo(a)pyrene and 2-acetylaminofluorene.** Eksp Onkol 1997;19(3):185-90.
BIOSIS COPYRIGHT: BIOL ABS. The mutagenic effect of a number of procarcinogenic compounds has been compared in two groups of Drosophila melanogaster strains differing in the insecticide resistance-associated cytochrome P-450. Heterozygotes y++/+wsn3 of insecticide-sensitive strains were shown to be sensitive to the mutagenic effects of aflatoxin B1, 1,2-dibromo-3-chloropropane and nitrozamines, but failed to detect mutagenicity of the benzo(a)pyrene and acethylaminofluorenes. In contrast, the newly established DDT-resistant strains were found to be sensitive to the mutagenecity of BP and 2-AAF in the SMART assay. Mechanisms of the sensitivity of the newly constructed strains to BP and 2-AAF are discussed.

Duthie SJ, Collins AR, Duthie GG, Dobson VL. **Quercetin and myricetin protect against hydrogen peroxide-induced DNA damage (strand breaks and oxidised pyrimidines) in human lymphocytes.** Mutat Res 1997;393(3):223-31.
The effects of the flavonoids quercetin and myricetin, and the antihepatotoxic agent silymarin, on hydrogen peroxide-mediated DNA damage in human lymphocytes were determined using alkaline single-cell gel electrophoresis (the comet assay). Treatment with hydrogen peroxide increased the levels of DNA strand breaks and oxidised pyrimidine bases in these cells. Quercetin was protective at concentrations above 10 microM and myricetin decreased oxidant-induced DNA strand breakage at concentrations of 100 microM. Cellular metabolism may alter the antioxidant efficacy of the flavonoids. Silymarin had no protective effect at any of the concentrations tested. None of these flavonoids was itself genotoxic. Neither alpha-tocopherol nor beta-carotene decreased hydrogen peroxide-induced DNA breakage. The differences in effectiveness of these dietary compounds against oxidative DNA damage may be explained by differences in their chemical structure or location within the cell.

Edenharder R, Rauscher R, Platt KL. **The inhibition by flavonoids of 2-amino-3-methylimidazo[4,5-f] quinoline metabolic activation to a mutagen: a structure-activity relationship study.** Mutat Res 1997;379(1):21-32.
The mutagenicity of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) in Salmonella typhimurium TA98 is inhibited by flavonoids with distinct structure-antimutagenicity relationships (Edenharder, R., I. von Petersdorff I. and R. Rauscher (1993). Antimutagenic effects of flavonoids, chalcones and structurally related compounds on the activity of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and other heterocyclic amine mutagens from cooked food, Mutation Res., 287, 261-274). With respect to the mechanism(s) of antimutagenicity, the following results were obtained here. (1) 7-Methoxy- and 7-ethoxyresorufin-O-dealkylase activities in rat liver microsomes, linked to cytochrome P-450-dependent 1A1 and 1A2 monooxygenases catalyzing oxidation of IQ to N-hydroxy-IQ (N-OH-IQ), were effectively inhibited by 16 flavonoids (IC50: 0.4-9.8 microM). Flavones and flavonols are in general more potent enzyme inhibitors than flavanones, isoflavones, and chalcones. Among flavones the presence of hydroxyl or methoxyl groups resulted in minor changes only. However, among flavonols and flavanones the parent compounds exerted the strongest inhibitory effects, which decreased in dependence on number and position of hydroxyl functions. Contrary to the results obtained in the Salmonella assay in the tests with alkoxyresorufins no extraordinary counteracting effects of

isoflavones, of hydroxyl groups at carbons 6 or 2' or of the elimination of ring B (benzylideneacetone) were detected. (2) No effects of flavonoids on NADPH-dependent cytochrome P-450 reductase activity could be detected. (3) The effects of 30 flavonoids on mutagenicity induced by N-OH-IQ in S. typhimurium TA98NR were again structure dependent. The most striking feature was the, in principle, reverse structure-antimutagenicity pattern as compared to IQ: non-polar compounds were inactive and a 50% inhibition was achieved only by some flavones and flavonols (IC50: 15.0-148 nmol/ml top agar). Within the flavone and flavonol subgroups inhibitory effects increased in dependence on number and position of hydroxyl functions. Isoflavones and flavanones, however, as well as glycosides, were inactive. Hydroxyl groups at carbons 7, 3', 4', and 5' generated antimutagenic compounds, a hydroxyl function at C5 was ineffective, but hydroxyls at C3 and 6 as well as methoxyl groups at C3' (isorhamnetin) or 4' (diosmetin) generated comutagenic compounds. 4. Cytosolic activation of IQ to mutagenic metabolites as determined by experiments with the hepatic S105 fraction comprises about 10% of the mutagenicity after activation by the combined microsomal and cytosolic fractions (S9). The pattern of inhibition as produced by 20 flavonoids was closely similar to that observed with the S9 fraction. 5. In various experiments designed for modulation of the mutagenic response, it could be shown that further mechanisms of flavonoid interaction with the overall mutagenic process may exist, such as interactions with biological membranes (luteolin, fisetin) and effects on fixation and expression of.DNA damage (flavone, fisetin).

El Mzibri M, Guiraud-Dauriac H, Laget M, Beudot C, De M M, Dumenil G. **Use of flow cytometry to detect genotoxins by the Salmonella sulA-test.** Biotechnol Tech 1997;11(7):467-70.
BIOSIS COPYRIGHT: BIOL ABS. The Salmonella sulA-test using Salmonella typhimurium TA1538/ pEM1968 (sulA::lacZ) is a new SOS-repair inducing system that detects mutagens and carcinogens. The beta-galactosidase activity, currently detected by colorimetric dosage, can be measured by flow cytometry using a fluorescent substrate (fluorescein-di-beta-D-galactopyranoside). Comparison of the dose-response relationships of eight chemicals determined by the two techniques showed that the Salmonella sulA-test combined with the flow cytometry technique was accurate and reliable as it covered a large number of cells in a short time.

Fahrig R. **Co-recombinogenic and co- or anti-mutagenic effects of non-genotoxic carcinogens in S. cerevisiae MP1.** J Environ Pathol Toxicol Oncol 1997;16(4):273-9.
BIOSIS COPYRIGHT: BIOL ABS. Carcinogens are generally classified into two groups: genotoxic and nongenotoxic. As the final product of genotoxic and non-genotoxic carcinogens is the same, that is, a clone of genetically altered cells, it could be possible that non-genotoxic carcinogens may yield genotoxic events as a secondary result of cell toxicity having led to mitogenesis/cellular proliferation, or that genetic alterations are induced that are normally neglected in genotoxicity tests. A genetic effect with possible relevance for the ultimate mechanism of carcinogenicity is recombination. In previous experiments using yeast, bacteria, Drosophila or mice, tumor promoters were co-recombinogenic/anti-mutagenic, and co-carcinogens were co-mutagenic/anti-recombinogenic. In previous in vivo-experiments using the spot test with mice, diethylhexylphthalate (DEHP) was co-recombinogenic and anti-mutagenic. In the present study with yeast, this specific type of genetic activity, co-recombinogenicity and anti-mutagenicity, was followed up. The reason for doing this was to see whether in an in vitro test with yeast similar results could be observed as in an in vivo test with mice. Beyond

DEHP, three other substances have been tested for comparison: formaldehyde as carcinogen with possibly non-genotoxic mode of action but activity in genotoxicity tests, acetamide as presumed non-genotoxic carcinogen with no data base in genotoxicity tests, and caprolactam as a noncarcinogen. With DEHP in combination with TEM (100% survival) an increase in recombination but also a clear co-mutagenic effect could be observed. In contrast to this, formaldehyde (100% survival) and acetamide (100 to 60% survival) were anti-mutagenic and co-recombinogenic. Caprolactam (100 to 65% survival) enhanced neither gene mutations nor recombination when given in combination with TEM.

Fink SP, Reddy GR, Marnett LJ. **Mutagenicity in Escherichia coli of the major DNA adduct derived from the endogenous mutagen malondialdehyde.** Proc Natl Acad Sci U S A 1997;94(16):8652-7.
The spectrum of mutations induced by the naturally occurring DNA adduct pyrimido[1,2-alpha]purin-10 (3H)-one (M1G) was determined by site-specific approaches using M13 vectors replicated in Escherichia coli. M1G was placed at position 6256 in the (-)-strand of M13MB102 by ligating the oligodeoxynucleotide 5'-GGT(M1G)TCCG-3' into a gapped-duplex derivative of the vector. Unmodified and M1G-modified genomes containing either a cytosine or thymine at position 6256 of the (+)-strand were transformed into repair-proficient and repair-deficient E. coli strains, and base pair substitutions were quantitated by hybridization analysis. Modified genomes containing a cytosine opposite M1G resulted in roughly equal numbers of M1G-->A and M1G-->T mutations with few M1G-->C mutations. The total mutation frequency was approximately 1%, which represents a 500-fold increase in mutations compared with unmodified M13MB102. Transformation of modified genomes containing a thymine opposite M1G allowed an estimate to be made of the ability of M1G to block replication. The (-)-strand was replicated >80% of the time in the unadducted genome but only 20% of the time when M1G was present. Correction of the mutation frequency for the strand bias of replication indicated that the actual frequency of mutations induced by M1G was 18%. Experiments using E. coli with different genetic backgrounds indicated that the SOS response enhances the mutagenicity of M1G and that M1G is a substrate for repair by the nucleotide excision repair complex. These studies indicate that M1G, which is present endogenously in DNA of healthy human beings, is a strong block to replication and an efficient premutagenic lesion.

Foltinova P, Grones J. **Euglena gracilis as an eukaryotic test organism for detecting mutagens and antimutagens.** Mutat Res 1997;393(1-2):1-6.
The unicellular flagellate Euglena gracilis was used in order to assess the inhibition of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and N-methyl-N-nitrosourea (MNU) mutagenicities, which induce white mutants due to the irreversible loss of chloroplasts. All tested compounds, including o-aminobenzoic acid and p-aminobenzoic acid, salicylic acid, acetylsalicylic acid, sodium salicylate and p-aminosalicylic acid, were not mutagenic per se and inhibited MNNG mutagenicity by at least 50%. The last two compounds inhibited by at least 50% also MNU mutagenicity.

Franzios G, Mirotsou M, Hatziapostolou E, Kral J, Scouras ZG, Mavragani-Tsipidou P. **Insecticidal and genotoxic activities of mint essential oils.** J Agric Food Chem 1997;45(7):2690-4.
BIOSIS COPYRIGHT: BIOL ABS. The essential oils (EOs) extracted from the mint species Mentha pulegium and Mentha spicata, together with their main constituents, pulegone, menthone, and carvone, were tested for insecticidal and genotoxic activities on Drosophila melanogaster. The EOs of both

aromatic plants showed strong insecticidal activity, while only the oil of M. spicata exhibited a mutagenic one. Among the constituents studied, the most effective insecticide was found to be pulegone, while the most effective for genotoxic activity was menthone. Data show that both toxic and genotoxic activities of the EOs of the two studied mint plants are not in accordance with those of their main constituents, pulegone, menthone, and carvone. Pulegone is significantly more effective (9 times) as an insecticide, while menthone and carvone are less effective (6 and 2 times, respectively) insecticides when used in their authentic forms, and a mixture of authentic pulegone and menthone, in levels resembling their content in the oil of M. pulegium, showed that the strong toxicity of pulegone is suppressed in the presence of menthone. All the above suggest that synergistic/antagonistic phenomena may be involved that alter the toxicity of the whole EO.

Giller S, Le Curieux F, Erb F, Marzin D. **Comparative genotoxicity of halogenated acetic acids found in drinking water.** Mutagenesis 1997;12(5):321-8.
Three short-term assays (SOS chromotest, Ames fluctuation test and newt micronucleus test) were performed to detect the genotoxic activity of organohalides, compounds likely to be found in chlorinated and/or ozonated drinking water: monochloro-, dichloro- and trichloroacetic acids and monobromo-, dibromo- and tribromoacetic acids. With the SOS chromotest, only three of the chemicals studied (dichloroacetic acid, dibromo- and tribromoacetic acids) were found to induce primary DNA damage in Escherichia coli PQ 37. In the Ames fluctuation test, all the compounds except monochloroacetic acid showed mutagenic activity in Salmonella typhimurium strain TA100. In these two in vitro tests, a good correlation between increasing number of substituents and decreasing mutagenicity was observed. Namely, the toxicity of brominated and chlorinated acetic acids decreased when the number of substituents increased. The newt micronucleus test detected a weak clastogenic effect on the peripheral blood erythrocytes of Pleurodeles waltl larvae for trichloroacetic acid only.

Hamilton CM, Dabbs JE, Cunningham GD, Vernetti LA, Mirsalis JC, Snyder RD. **Evaluation of positive controls for the in vitro unscheduled DNA synthesis assay using hepatocytes from induced (Aroclor 1254) and uninduced male cynomolgus monkey.** Environ Mol Mutagen 1997;30(3):354-8.
We have evaluated the use of four different positive control compounds for assessing UDS in monkey hepatocytes and have found three of these, methylmethanesulfonate, benzo[a]pyrene, and dimethylbenz [a]anthracene, to produce strong positive responses in vitro. Dimethylnitrosamine induced only weak responses. We also report that the strength of the response induced by procarcinogens was not enhanced in hepatocytes taken from Aroclor 1254-pretreated monkeys, even though substantial induction of cytochrome P450 enzymes was demonstrated in these cells. These studies raise the question of the utility of employing an in vivo induction system to enhance the monkey UDS assay.

Imaoka S, Yoneda Y, Matsuda T, Degawa M, Fukushima S, Funae Y. **Mutagenic activation of urinary bladder carcinogens by CYP4B1 and the presence of CYP4B1 in bladder mucosa.** Biochem Pharmacol 1997;54(6):677-83.
BIOSIS COPYRIGHT: BIOL ABS. We investigated the mutagenic activation of 2-naphthylamine (2-NA), 3,2'-dimethyl-4-aminobiphenyl (DMAB), and 3,3'-dichlorobenzidine (DCB), bladder carcinogens, by renal and bladder microsomes and by purified P450s using the umu gene expression system, which detects DNA damage. Mouse renal microsomes had high mutagenic activation toward DCB and low

activity toward 2-NA. Purified mouse Cyp4b1 also had high mutagenic activity toward DCB. Anti-Cyp4b1 antibody efficiently inhibited DCB bioactivation by mouse renal microsomes with a high Cyp4b1 content. Lauric acid, a substrate of Cyp4b1, efficiently inhibited DCB bioactivation by renal and bladder microsomes of the mouse and by purified Cyp4b1. To assess the contribution of CYP4B1 to bladder carcinoma, further investigation was done with the umu test and an immunochemical study. Ten forms of purified rat P450s including rat CYP4B1 were used in the umu test for 2-NA, DMAB, and DCB. CYP4B1 had the highest activity toward DMAB and DCB. Other P450s had activities of less than 20% that of CYP4B1. CYP4B1 also activated 2-NA, but its activity was about 10% of that toward DMAB or DCB. Rat bladder epithelium was stained specifically with anti-Cyp4b1 antibody, indicating the presence of CYP4B1 in the rat bladder mucosa. Also, CYP4B1 mRNA was detected by northern blotting add reverse transcription-polymerase chain reaction (RT-PCR). These findings suggested that CYP4B1 could contribute to the initiation of carcinogenesis in rat and mouse bladder by activation of aromatic amines.

Johnston PJ, Stoppard E, Bryant PE. **Induction and distribution of damage in CHO-K1 and the X-ray-sensitive hamster cell line xrs5, measured by the cytochalasin-B-cytokinesis block micronucleus assay.** Mutat Res 1997;385(1):1-12.
The micronucleus assay holds promise as a method for determining clastogenic effects of particular agents and for examining relative sensitivities of eukaryotic cells to such clastogens. In the following work, a detailed examination of the induction of micronuclei in radio-resistant Chinese hamster ovary fibroblasts (CHO-K1) and the DNA double-strand break repair-defective daughter cell line, xrs5, was performed. Cells were exposed to gamma-irradiation, bleomycin, etoposide, camptothecin and the restriction endonuclease PvuII. By a simplified statistical analysis of data, information on the expression of chromosomal damage, the distribution of damage and the role of cell cycle effects on damage expression was obtained from a relatively small number of cells. All 5 clastogens resulted in elevated levels of micronuclei in xrs5 compared to CHO-K1. An analysis of the distribution of micronuclei within treated populations revealed differences between the modes of damage. Significant deviation from the expected values indicated that expression of micronuclei does not follow an expected Poisson distribution. The frequencies of binucleated cells indicated micronucleus frequencies do not always correlate with inhibition of cell cycle progression. This work also demonstrates that caution is required in the interpretation of data obtained through micronucleus assays. In particular, it does not appear possible to proscribe simple numerical values of relative sensitivity or clastogenicity based on the relative number of micronuclei induced alone.

Karamova NS, Il'inskaia ON, Ivanchenko OB, Ermolaev AI, Gil'mutdinov GZ, Gershanov FB, Khamitov BR. **[Genotoxic effects of tonarol]**. Genetika 1997;33(9):1310-2. (Rus)
Genotoxic effects of 2,6-di-tret-butyl-4-methylphenol (tonarol) were studied using four test systems: the Ames test, the SOS chromotest, the cytogenetic test with rootlets of onion (Allium cepa), and the in vivo micronucleus test. Tonarol did not affect gene mutation induction in Salmonella typhimurium tester strains, the SOS response in the Escherichia coli strain PQ37, chromosomal aberrations in cells of onion (Allium cepa) rootlets, and micronuclei in erythrocytes of peripheral blood of CBA x C5713 L/G mice. Tonarol induced cell division in A.

Kornuta N, Bagley E, Nedopitanskaya N. **Genotoxic effects of pesticides.** J Environ Pathol Toxicol Oncol 1996;15(2-4):75-8.
BIOSIS COPYRIGHT: BIOL ABS. Epidemiologic data showed an increase in the number of cancer cases in persons involved in agricultural production using pesticides. According to IARC, more than 25% of pesticides are classified as oncogens. In recent years, the concept of malignant tumors developing after environmental contamination with chemicals has been accepted. Changes in genetic material are at the basis of this process because many environmental pollutants are chemical carcinogens and mutagens with the capacity of causing DNA damage. DNA damage was proposed as a useful parameter for assessing the genotoxic properties of environmental pollutants. The correlation between exposure to carcinogenic substance and the level of DNA damage is essential. Pesticides are highly biologically active chemicals. They may interact with DNA and damage its structure. Such interaction may be critical for the manifestation of carcinogenic properties of different chemicals. We report on the organotropic genotoxic effects of different chemical classes of pesticides (decis, cypermetrin, 2,4-D, polyram) studied by means of alkaline unwinding assay DNA.

Kranendonk M, Commandeur JN, Laires A, Rueff J, Vermeulen NP. **Characterization of enzyme activities and cofactors involved in bioactivation and bioinactivation of chemical carcinogens in the tester strains Escherichia coli K12 MX100 and Salmonella typhimurium LT2 TA100.** Mutagenesis 1997;12(4):245-54.
MX100 is an Escherichia coli K12 genotoxicity tester strain, especially developed for mechanistic studies of chemical mutagens and carcinogens. For the study of the role of specific enzymes in the bioactivation and bioinactivation of carcinogens, it is necessary to characterize MX100 as far as its metabolic bio(in)activation capacities are concerned. In this study such a characterization is performed in two types of cell-free lysates, one derived from stationary phase cells, grown in rich medium (SR-lysates) and one from exponentially growing cells (log phase), cultured in minimal medium (LM-lysates). Six Phase I enzyme activities of aromatic NADPH hydroxylase, NADH hydroxylase, flavin-containing monooxygenase (FMO), nitroreductase, DT-diaphorase and NADPH ferredoxin: oxidoreductase were determined. Activities of six Phase II enzymes glutathione S-transferases (GSTs), N-aryl acetyltransferase (NAT), arylamine sulphotransferase, UDP-glucuronyltransferase and epoxide hydratase and of the Phase III enzyme cysteine conjugate beta-lyase were subsequently assessed. In addition, five antioxidant enzymes: superoxide dismutase (SOD), catalase, glutathione (GSH)-reductase, GSH-peroxidase and alkyl hydroperoxide reductase; as well as concentrations of glutathione (GSH) and its disulphide (GSSG), were measured. The activity parameters of all enzymes were compared with those obtained in similar lysates of the Salmonella strain TA100 and in rat liver preparations. The results indicate that MX100 as well as TA100 contain relatively low oxidative but high reductase Phase I activities. Both strains demonstrated low activities for the Phase II conjugation enzymes except for GSTs. In MX100, relatively high activities were detected for all antioxidative enzymes, activities which were lower in TA100. Significant differences in activities were observed between the SR-lysates derived from stationary phase/rich medium and LM-lysates from log phase/minimal medium cells for nitroreductase, GST, SOD, catalase, NADPH ferredoxin:oxidoreductase as well as in GSH content. In general, we described for the first time a metabolic characterization of the E.coli tester strain MX100 and the Salmonella typhimurium strain TA100 and discussed the results in terms of its significance for carcinogen bioactivation and bioinactivation capacities.

Le Curieux F, Munter T, Kronberg L. **Identification of adenine adducts formed in reaction of calf thymus DNA with mutagenic chlorohydroxyfuranones found in drinking water.** Chem Res Toxicol 1997;10(10):1180-5.
BIOSIS COPYRIGHT: BIOL ABS. Calf thymus DNA was reacted with the extremely potent bacterial mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) and the structurally related compounds 3,4-dichloro-5-hydroxy-2(5H)-furanone (MCA) and 3-chloro-4-methyl-5-hydroxy-2(5H)-furanone (MCF). The chromatograms of the HPLC analyses of the DNA hydrolysates showed peaks that represented adducted base moieties. It was possible to establish the structures of the adducts by comparing UV spectra and chromatographical properties of the DNA adducts with known adenosine and 2'-deoxyadenosine adducts. The DNA adduct produced by MX was identified as 3-(2'-deoxyribofuranosyl-N6-adenosinyl)propenal (M1A-dR). It was calculated that 1 nucleotide/105 nucleotides was converted to M1A-dR. The same adduct was formed also in the reaction of MX with 2'-deoxyadenosine (yield 0.01%). The M1A-dR adduct may play a role in the mutational events induced by MX in Salmonella typhimurium strain TP2428. The adducts produced in the reactions of MCA and MCF with DNA were identified as 3-(2'-deoxyribofuranosyl)-7-formylimidazo(2,1-i)purine (epsiloncA-dR) and 4-(2'-deoxyribofuranosyl-N6-adenosinyl)-3-formyl-3-butenoic acid (fbaA-dR), respectively. The yield of epsiloncA-dR was 5 adducts/106 nucleotides and of fbaA-dR 4 adducts/105 nucleotides. The biological significance of these adducts is unknown.

Leung HW, Ballantyne B. **Evaluation of the genotoxic potential of alkylalkanolamines.** Mutat Res 1997;393(1-2):7-15. Three alkylalkanolamines, N,N-dimethylethanolamine, N-methyldiethanolamine, and tert-butyldiethanolamine, were evaluated for potential genotoxic activity using the Salmonella/microsome reverse gene mutation test, the CHO/HGPRT forward gene mutation test, a sister chromatid exchange test in cultured CHO cells, and an in vivo peripheral blood micronucleus test in Swiss-Webster mice. None of the three alkylalkanolamines produced any significant or dose-related increases in the frequencies of mutations, sister chromatid exchanges or micronuclei. These results indicate that N,N-dimethylethanolamine, N-methyldiethanolamine, and tert-butyldiethanolamine are not genotoxic in the tests conducted.

Little JB, Nagasawa H, Pfenning T, Vetrovs H. **Radiation-induced genomic instability: delayed mutagenic and cytogenetic effects of X rays and alpha particles.** Radiat Res 1997;148(4):299-307. The frequency of mutations at the Hprt locus was measured in clonal populations of Chinese hamster ovary cells derived from single cells surviving exposure to 0-12 Gy of X rays or 2 Gy of alpha particles. Approximately 8-9% of 446 clonal populations examined 23 population doublings after irradiation showed high frequencies of late-arising mutations as indicated by mutant fractions 10(2)-10(4)-fold above background. The frequency with which such clones occurred was similar for alpha-particle irradiation and X irradiation, with no apparent dose dependence for X irradiation over the range of 4-12 Gy. The molecular structure of Hprt mutations was determined by analysis by multiplex polymerase chain reaction of all nine exons. Of mutations induced directly after exposure to X rays, 75% involved partial or total gene deletions. Only 19-23% of late-arising (delayed) mutations were associated with deletions, the preponderance of these being partial deletions involving one or two exons. This spectrum was very similar to that for spontaneously arising mutations. To determine whether delayed mutations

were non-clonal, the spectrum of exons deleted was examined among 29 mutants with partial deletions derived from a single clonal population. The results indicated that at least 15 of these mutants arose independently. To examine the relationship between the occurrence of delayed mutations and chromosomal instability, 60 Hprt mutant subclones isolated from a clonal population showing a high frequency of delayed mutations were serially cultivated in vitro. Of these, 14 showed a slow-growth phenotype with a high frequency of polyploid cells (10-38%) and a markedly enhanced frequency of non-clonal chromosomal rearrangements including both chromosome-type and chromatid-type aberrations. These clones also showed a 3- to 30-fold increase in the frequency of ouabain-resistant mutations; no ouabain-resistant mutants were induced directly by X irradiation. These results suggest that among clones showing a high frequency of delayed mutations there may be a subpopulation of cells that are particularly unstable; selection for the slow-growth phenotype has the effect of selecting for this chromosomally unstable subpopulation.

Liu N, Lamerdin JE, Tucker JD, Zhou ZQ, Walter CA, Albala JS, Busch DB, Thompson LH. **The human XRCC9 gene corrects chromosomal instability and mutagen sensitivities in CHO UV40 cells.** Proc Natl Acad Sci U S A 1997;94(17):9232-7.
The Chinese hamster ovary (CHO) mutant UV40 cell line is hypersensitive to UV and ionizing radiation, simple alkylating agents, and DNA cross-linking agents. The mutant cells also have a high level of spontaneous chromosomal aberrations and 3-fold elevated sister chromatid exchange. We cloned and sequenced a human cDNA, designated XRCC9, that partially corrected the hypersensitivity of UV40 to mitomycin C, cisplatin, ethyl methanesulfonate, UV, and gamma-radiation. The spontaneous chromosomal aberrations in XRCC9 cDNA transformants were almost fully corrected whereas sister chromatid exchanges were unchanged. The XRCC9 genomic sequence was cloned and mapped to chromosome 9p13. The translated XRCC9 sequence of 622 amino acids has no similarity with known proteins. The 2.5-kb XRCC9 mRNA seen in the parental cells was undetectable in UV40 cells. The mRNA levels in testis were up to 10-fold higher compared with other human tissues and up to 100-fold higher compared with other baboon tissues. XRCC9 is a candidate tumor suppressor gene that might operate in a postreplication repair or a cell cycle checkpoint function.

Liu S, Dixon K. **Induction of mutagenic DNA damage by chromium(VI) and glutathione**. Environ Mol Mutagen 1996;28(2):71-9.
The role of chromium (7440473) induced DNA damage in chromium mutagenicity was studied in-vitro. Plasmid pZ189 DNA was incubated with 20 micromolar (microM) potassium-dichromate (7778509) as a source of hexavalent chromium (Cr6+) or chromic-chloride (10025737) as a source of trivalent chromium (Cr3+) in the presence or absence of 100microM glutathione (GSH). Induction of DNA damage was assessed by measuring the extent of formation of DNA nicks using a gel electrophoresis technique. Cr6+ plus GSH caused a significant induction of DNA damage. Neither Cr6+ or GSH alone, nor Cr3+ with or without GSH induced DNA damage. Plasmid pZ189 DNA was incubated with up to 20microM Cr6+ or Cr3+ in the presence of a five molar excess of GSH. Plasmid biological activity was assessed by transferring the treated DNA into Escherichia-coli-MBM7070 and scoring the cells for transformed colonies. Mutagenicity was evaluated by transfecting CV-1 cells, the TC-7 clone of African-green-monkey kidney cells, with the treated pZ189 plasmid, removing the plasmid 48 hours later, incorporating the plasmid into MBM7070, and counting the number of white, light blue, and blue

colored cells after treatment with isopropyl-beta-D-thiogalactoside, 5-bromo-4-chloro-3-indoyl-beta-D-galactoside, and ampicillin. C6+ plus GSH caused a dose related increase in DNA damage and decreases in plasmid biological activity. Cr3+ and GSH did not produce DNA damage or alter plasmid biological activity. Combined Cr6+ and GSH treatment caused a significant increase in mutant white and light blue cells. The Cr3+ and GSH combination was not mutagenic. An electrophoretic analysis of the mutant plasmid DNA was performed. Of the mutants, 43% were deletions, mostly guanine/cytidine to adenine/thymidine (GC/AT) transitions, and the remainder were base substitutions, mostly GC/AT and guanine/cytidine to cytidine/guanine transversions. The authors conclude that Cr6+ and GSH can induce premutagenic DNA damage in-vitro. The mutant pattern is similar to that observed with other agents that cause oxidative DNA damage in that a significant number of deletions and base substitutions are produced. Cr3+ does not induce premutagenic or lethal DNA damage.

Lowcock LA, Sharbel TF, Bonin J, Ouellet M, Rodrigue J, Desgranges JL. **Flow cytometric assay for in vivo genotoxic effects of pesticides in green frogs (Rana clamitans).** Aquatic Toxicol 1997;38 (4):241-55.
BIOSIS COPYRIGHT: BIOL ABS. Frogs from farming regions in Quebec suffer a suite of physical and physiological problems associated with the use of agricultural pesticides. Flow cytometry was used to compare incidence of abnormal DNA profile, half-peak coefficient of variation (CV), and variation in genome size (pg DNA per haploid nucleus) between Green frogs (Rana clamitans) from such farming areas (corn and potato fields) and control sites dissociated from agricultural practices, to infer possible genomic manifestations of pesticide use. There was a significant ($P<0.05$). In all comparisons, adult frogs showed greater CVs than did juveniles ($P<0.0001$). Among adults, CVs were higher for samples taken from both potato and corn fields relative to the control samples ($P < 0.005$), while in juveniles, only individuals from corn plots showed elevated CVs relative to controls ($P<0.05$). Juveniles showing physical deformity had significantly higher CVs than normal individuals ($P< 0.05$), although there were no similar correlations with physiological disruption. Mean C-value (variation in DNA content) was different between adults and juveniles in all treatments ($P < 0.0001$), but there were no significant differences in mean C-value and variance of such among similar age classes between treatments. The different classes of DNA damage found in this study are reflective of either acute or cumulative pesticide toxicity, and are exhibited by both sick and apparently normal individuals. We therefore believe flow cytometry to be a powerful technique for the measurement of pesticide-induced genomic disruption in amphibian populations.

Mabon N, Moorthy B, Randerath E, Randerath K. **Monophosphate 32P-postlabeling assay of DNA adducts from 1,2:3,4-diepoxybutane, the most genotoxic metabolite of 1,3-butadiene: in vitro methodological studies and in vivo dosimetry**. Mutat Res 1996;371(1-2):87-104.
Methods for the detection and measurement of 1,2:3,4-diepoxybutane (1464535) (BDE) DNA adducts were described. A dinucleotide/monophosphate version of the phosphorus-32 (P32) postlabeling assay was used for the detection of calf thymus DNA modified by BDE in-vitro and for the determination of BDE/DNA adducts formed in-vivo following topical treatment of the shaved dorsal skin of ICR-mice with BDE. Optimal conditions for the detection of BDE adducts using the modified P32 postlabeling assay were described. Similar adduct patterns containing four major fractions were identified in in-vitro and in-vivo BDE modified samples. One of the in-vivo BDE fractions contained a fast moving adduct

which was not present in in-vitro modified or control DNA. Autoradiographic comparison of BDE modified oligonucleotides of different sequences and BDE modified calf thymus DNA suggested that the major BDE induced adduct fractions were derived from adenine and to a smaller extent from cytosine. The modified P32 postlabeling assay was found to have a linear BDE dose response; the in-vivo formation of BDE/DNA adducts was dose dependent as well. The limit of detection of the assay was about 1 adduct/10(8) unmodified nucleotides. The authors conclude that the assay described may be useful in determinations of BDE related adduct formation as well as adducts induced by other polar epoxides.

Maes A, Collier M, Van Gorp U, Vandoninck S, Verschaeve L. **Cytogenetic effects of 935.2-MHz (GSM) microwaves alone and in combination with mitomycin C.** Mutat Res 1997;393(1-2):151-6. This paper focuses on the genetic effects of microwaves from mobile communication frequencies (935.2 MHz) alone and in combination with a chemical DNA-damaging agent (mitomycin C). Three cytogenetic endpoints were investigated after in vitro exposure of human whole blood cells. These endpoints were the 'classical' chromosome aberration test, the sister chromatid exchange test and the alkaline comet assay. No direct cytogenetic effect was found. The combined exposure of the cells to the radiofrequency fields followed by their cultivation in the presence of mitomycin C revealed a very weak effect when compared to cells exposed to mitomycin C alone.

Malyapa RS, Ahern EW, Straube WL, Moros EG, Pickard WF, Roti Roti JL. **Measurement of DNA damage after exposure to 2450 MHz electromagnetic radiation.** Radiat Res 1997;148(6):608-17. Recent reports suggest that exposure to 2450 MHz electromagnetic radiation causes DNA single-strand breaks (SSBs) and double-strand breaks (DSBs) in cells of rat brain irradiated in vivo (Lai and Singh, Bioelectromagnetics 16, 207-210, 1995; Int. J. Radiat. Biol. 69, 513-521, 1996). Therefore, we endeavored to determine if exposure of cultured mammalian cells in vitro to 2450 MHz radiation causes DNA damage. The alkaline comet assay (single-cell gel electrophoresis), which is reportedly the most sensitive method to assay DNA damage in individual cells, was used to measure DNA damage after in vitro 2450 MHz irradiation. Exponentially growing U87MG and C3H 10T1/2 cells were exposed to 2450 MHz continuous-wave (CW) radiation in specially designed radial transmission lines (RTLs) that provided relatively uniform microwave exposure. Specific absorption rates (SARs) were calculated to be 0.7 and 1.9 W/kg. Temperatures in the RTLs were measured in real time and were maintained at 37 +/- 0.3 degrees C. Every experiment included sham exposure(s) in an RTL. Cells were irradiated for 2 h, 2 h followed by a 4-h incubation at 37 degrees C in an incubator, 4 h and 24 h. After these treatments samples were subjected to the alkaline comet assay as described by Olive et al. (Exp. Cell Res. 198, 259-267, 1992). Images of comets were digitized and analyzed using a PC-based image analysis system, and the normalized comet moment and comet length were determined. No significant differences were observed between the test group and the controls after exposure to 2450 MHz CW irradiation. Thus 2450 MHz irradiation does not appear to cause DNA damage in cultured mammalian cells under these exposure conditions as measured by this assay.

Marzin D. **The position of the in vitro micronucleus test within the battery of screening for genotoxic potential determination and the regulatory guidelines.** Mutat Res 1997;392(1-2):175-81. The in vitro micronucleus test is available to demonstrate the clastogenic and aneugenic potencies of

chemicals. This test is easier to evaluate than metaphase analysis and, although used routinely by some laboratories as a prescreening test for the determination of chromosomal mutation induction potency, it has not been recognised by regulatory authorities as a test to be included in the battery of assays for human risk assessment. This limitation is due to the lack of a fixed protocol, a limited data bank, particularly in the case of clastogens, and to the lack of sufficient robust interlaboratory validation studies. If we aim to recognize this test in the form of an OECD guideline and to introduce it as an alternative to in vitro metaphase analysis in cell culture, it is necessary to begin a collaborative study in order to determine the best protocol which is valid for the detection of all clastogens as well as to determine clearly the limits of the test. The use of this in vitro test as a prescreening assay could be very useful as it takes structural and numerical chromosomal abnormalities into account and is simpler to perform than in vitro metaphase analysis.

Matas N, Thygesen P, Stacey M, Risch A, Sim E. **Mapping AAC1, AAC2 and AACP, the genes for arylamine N-acetyltransferases, carcinogen metabolising enzymes on human chromosome 8p22, a region frequently deleted in tumours.** Cytogenet Cell Genet 1997;77(3-4):290-5.
Arylamine N-acetyltransferases (NATs) are encoded at two loci on 8p22, a region subject to deletions in bladder tumours. The two functional genes (AAC1 and AAC2 alias NAT1 and NAT2) without introns in the coding region, encode enzymes which metabolise carcinogens, including bladder carcinogens. They are both multi-allelic and certain alleles have been implicated as susceptibility factors in bladder cancer. There is a third N-acetyltransferase gene, a pseudogene, AACP alias NATP, which we show is also located on chromosome 8 at the p22 region. We have mapped a series of YAC clones (ICI and CEPH) containing the NAT genes and the markers D8S21, an RFLP marker, and D8S261, a microsatellite marker. We show that D8S21 is a portion of the coding region of AAC2. The order of genes in this region, covering some 2 Mb, is TEL-D8S261-AAC1-AACP-AAC2 (D8S21)-CEN. The restriction map also illustrates that there are likely to be other expressed genes in the region through the identification of CpG islands.

McHugh PJ, Knowland J. **Characterization of DNA damage inflicted by free radicals from a mutagenic sunscreen ingredient and its location using an in vitro genetic reversion assay.** Photochem Photobiol 1997;66(2):276-81.
We describe an in vitro approach to assessing the potential genotoxicity of illuminated sunscreens. The photomutagenic sunscreen Padimate-O attacks DNA on illumination with simulated sunlight, producing strand breaks and lesions that are labile to N,N'-dimethylethylenediamine but few, if any, cyclobutane dimers or other direct photoproducts. The damage can be completely suppressed by the free radical quenchers Tris, ethanol, mannitol and dimethylsulfoxide, which is commonly used as a solvent in conventional photomutagenicity assays. Using a genetic reversion assay that depends on regenerating beta-galactosidase activity in photodamaged plasmids we find that GC base pairs are particularly susceptible to attack by Padimate-O.

Menichini P, Viaggi S, Gallerani E, Fronza G, Ottaggio L, Comes A, Ellwart JW, Abbondandolo A. **A gene trap approach to isolate mammalian genes involved in the cellular response to genotoxic stress.** Nucleic Acids Res 1997;25(23):4803-7.
Treatment of cells with DNA damaging agents leads to induction of a variety of genes involved in

different cellular processes. We have applied a lacZ-based gene trap strategy to search for new mammalian genes induced by genotoxic stress. A population of 32 x 10(3) neo r clones stably transfected with a gene trap vector was obtained, stained with fluorescein di-beta-d-galactopyranoside and analyzed by flow activated cell sorting and replica plating. This strategy allowed isolation of 30 neo r 'putative inducible' cell lines expressing lacZ only after a DNA damaging treatment. For three clones the site of integration and the degree of inducibility after UV treatment were determined by Southern blot and beta-galactosidase measurement respectively. One cell line (clone VI) showed a single integration site and a reproducible 3-fold induction of beta-galactosidase activity following UV irradiation. Fused transcripts were isolated from induced cells and a portion of the trapped gene was amplified by rapid amplification of cDNA ends. Sequence analysis and comparison with available gene and protein databanks revealed that the gene was novel.

Miadokova E, Macakova K, Podstavkova S, Vlcek D. **Genotoxic properties of the newly synthesized antineoplastic agents amidox, didox, and trimidox**. Pharmazie 1997;52(7):540-4.
CBAC COPYRIGHT: CHEM ABS Toxic and genotoxic effects of 3 polyhydroxy-substituted benzohydroxamates (amidox, didox, and trimidox), having antineoplastic activities by the mechanism of the ribonucleotide reductase activity inhibition, were evaluated by reverse mutation assay on Salmonella typhimurium strains TA97, TA98, TA100, TA102. While amidox did not exert any toxic effect, didox, and trimidox were toxic. The toxicity of the test chems. was dependent on the structure of their mol. and the repair capacity of the test strains. Trimidox exhibited the highest toxicity, and it was proved as a direct-acting frameshift mutagen. Its mutagenic effect was increased after a metabolic activation. Amidox and didox can be classified as frameshift promutagens.

Mihara Y, Doi M, Inohara T, Kawamura M, Hamanaka N, Ishida T. **Interaction of mutagenic tryptophan pyrolysate with d(CGATCG)2: intercalation model based on NMR experiments.** Biochem Biophys Res Commun 1997;240(3):803-6.
The solution structure of the complex of 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1), a potent mutacarcinogen isolated from tryptophan pyrolysate, with the hexamer duplex d(CGATCG)2, was analysed by 1H-NMR spectroscopy and molecular dynamic calculation. Trp-P-1 was intercalated between the CpG base pairings in a suitable manner to form the guanosine-Trp-P-1 adduct which corresponds to the major reactant of Trp-P-1 with DNA.

Miller B, Albertini S, Locher F, Thybaud V, Lorge E. **Comparative evaluation of the in vitro micronucleus test and the in vitro chromosome aberration test: industrial experience.** Mutat Res 1997;392(1-2):45-59, 187-208.
Because of its rapidness, simplicity and potential for automation, the measurement of micronucleated cells in vivo is not only equivalent to the analysis of chromosome aberrations, but often even preferred within routine genotoxicity testing. In order to evaluate the correlation between the in vitro micronucleus assay (MNT) and the in vitro chromosome aberration test (CA), we collected data from four pharmaceutical companies obtained either in Chinese hamster cell lines (CHO-K5, CHO-K1, V79) or in human peripheral blood lymphocytes. Among the 57 compounds included in this comparison, 45 compounds gave rise to concordant results in both assays (26 compounds negative in both assays; 19 compounds positive in both assays). The high percentage of concordance, i.e. about 79% is very

promising and can be even increased to about 88% by omitting the 3 aneugenic compounds and 2 compounds inducing endoreduplicated chromosomes which were found positive only in the in vitro MNT. The results are remarkable in particular considering that most of the compounds evaluated are 'standard' pharmaceutical compounds and thus are at most weak inducers of chromosome damage. Our comparison strongly supports that the in vitro micronucleus test is a suitable alternative to the in vitro chromosome aberration assay. Moreover, the MNT has the potential of not only detecting clastogens but additionally aneuploidy inducing chemicals.

Miyamae Y, Iwasaki K, Kinae N, Tsuda S, Murakami M, Tanaka M, Sasaki YF. **Detection of DNA lesions induced by chemical mutagens using the single-cell gel electrophoresis (comet) assay. 2. Relationship between DNA migration and alkaline condition.** Mutat Res 1997;393(1-2):107-13.
The alkaline condition is an important factor for the alkaline single-cell gel electrophoresis (SCG) assay to detect the genotoxic effects of chemicals. In order to understand the relationship between DNA migration and alkaline condition, the effect of 13 model chemical mutagens with different modes of action was evaluated with the alkaline SCG assay under two different alkaline conditions (pH 12.1 and 12.6). CHO cells were sampled just after treatment for 1 h. The X-ray mimetic mutagen BLM increased DNA migration at pH 12.1 and 12.6 and the results were the same at both pH values. Six alkylating mutagens MNU, ENU, MNNG, ENNG, MMS, and EMS and one base adduct inducer 4-NQO induced a dose-dependent response only at pH 12.6. Two DNA crosslinking agents, MMC and DDP, and AMD had negative results. MMC and DDP, however, reduced the positive response of BLM, suggesting that DNA crosslinks could be detected. These results demonstrated that the alkaline condition was important factor for the alkaline SCG assay to detect the genotoxic effects of chemicals.

Miyamae Y, Zaizen K, Ohara K, Mine Y, Sasaki YF. **Detection of DNA lesions induced by chemical mutagens by the single cell gel electrophoresis (comet) assay. 1. Relationship between the onset of DNA damage and the characteristics of mutagens.** Mutat Res 1997;393(1-2):99-106.
We evaluated the relationship between the onset of DNA damage and the characteristics of 5 model chemical mutagens with the single-cell gel electrophoresis (SCG) assay using L5178Y mouse lymphoma cells. We treated the cells with each chemical for 3 h and sampled them 0, 21, and 45 h after treatment. DNA damage induced by UV mimetic mutagens MMS and MNU, and X-ray mimetic mutagen BLM was observed just after treatment, crosslinking agent MMC-induced DNA damage was detected 21 h after treatment, and 6-MP as an inhibitor of DNA synthesis did not induce DNA damage at any sampling time. These results suggest that the SCG assay detects DNA lesions just after treatment with UV and X-ray mimetic mutagens, but needs a waiting period after treatment with crosslinking agents.

Morales-Ramirez P, Vallarino-Kelly T, Anguiano-Orozco G, Rodriguez-Reyes R. **Pharmacokinetic parameters of genotoxic activity inferred from the comparison of the kinetics of MN-PCE induced by chemical agents and ionizing radiation.** Mutat Res 1997;391(3):127-34.
Some kinetic parameters of clastogenic activity of cyclophosphamide were inferred by means of the comparison of its kinetics of micronucleated polychromatic erythrocytes (MN-PCE) formation with the kinetics induced by radiation. The same reasoning was also applied to the kinetics obtained by treatment with mitomycin C (MMC), arabinocyl cytosine (Ara-C) and 6-mercaptopurine (6-MOP), based on

previously reported data from the literature. The results indicate that the latency period (LP) and half-lives (HL) vary from one mutagen to another. For MMC, they are very similar to radiation indicating a rapid distribution and reaction. CP presents very long LP and HL which agree with the requirement of mutagen activation. Ara-C showed a very short LP which suggests a rapid activation and fast induction of damage in DNA. 6-MOP presented a very long LP which agreed with the requirement of its incorporation into DNA to cause micronucleus (MN). From the data obtained in the present work, it can be concluded that the comparison of the kinetics of MN-PCE formation induced by chemical agents with that obtained by the exposure to an acute dose of radiation permits one to estimate some parameters of the kinetics of clastogenic activity of chemical agents, like the LP and the HL. This seems to be valid for agents that act through the induction of DNA lesions; in the case of agents whose clastogenic activity is through other mechanisms, such as the inhibition or alteration of the process of duplication of the DNA, the kinetic parameters are not equivalent to the LP and HL; however, they could provide information on their possible mechanism of action.

Nielsen PS. **Bulky carcinogen-DNA adducts and exposure to environmental and occupational sources of polycyclic aromatic hydrocarbons. Influence of susceptibility genotypes on adduct level [doctoral thesis].** Aarhus (Denmark): University of Aarhus; 1996. 97 P.
The purpose of this doctoral thesis was to develop the use of markers of biological effective dose in polycyclic aromatic hydrocarbon (PAH) exposure assessment. Specifically, the feasibility of the phosphorus-32 postlabeling method to detect carcinogen-DNA adducts was addressed. A second aim was to evaluate the influence of polymorphism in metabolizing enzyme genes on DNA adduct levels. Third, an effort was made to establish some kind of baseline DNA adduct level for individuals with supposed low exposure, and compare it to the more exposed groups. Fourth, an attempt was made to examine whether biomarkers of genotoxic exposure could be used in epidemiological studies to identify risk groups and contribute with better exposure estimates to the study of PAH related cancer risk. The author concludes tentatively that in estimation of exposure to PAH, inhalation should not be the only route of absorption considered. Biomarkers such as DNA adducts represent the total absorbed dose from all exposure routes in which skin absorption may be the most important one. A significant difference was demonstrated in DNA adduct levels between urban and rural exposed groups, with relatively low exposure differences in air pollution. DNA adduct levels were demonstrated to indicate a distinction between different exposure levels in a potential uniformly exposed group of bus drivers. It was noted that a clear, but not significant, trend exists for the influence of susceptibility genotype GSTM1 on adduct levels. For rural and urban exposure, a baseline level of bulky DNA adducts was established.

Nilsson R, Nordlinder R, Hogstedt B, Karlsson A, Jarvholm B. **Symptoms, lung and liver function, blood counts, and genotoxic effects in coastal tanker crews.** Int Arch Occup Environ Health 1997;69 (6):392-8.
OBJECTIVE: The deck crew on tankers can be exposed to high concentrations of benzene and other chemicals during loading, unloading and tank-cleaning operations. The objective of this study was to investigate whether genotoxic or other early health effects of cargo vapour exposure could be detected in coastal tanker crews. METHODS: The association between exposure to cargo vapours and clinical symptoms and signs, spirometry, blood cell count, blood test for liver function, and the frequency of micronuclei and sister chromatid exchanges in peripheral lymphocytes was studied in a cross-sectional

investigation of 107 male crew members (66 deck crew and 41 others) on ten coastal tankers. RESULTS: Seven of the tankers had automatic cargo level gauging systems but some of the ships still had open hatches during loading and unloading operations. Acute symptoms such as headache, nausea, vertigo, fatigue and dizziness after loading or tank-cleaning operations were reported by 56 of the 66 deck crew members (85%). Irritation of the mucous membrane in eyes and upper respiratory tract by cargo vapours were also common in this group. Obstructive symptoms were more common in the group with the highest exposure to cargo vapours but persistent effects on lung function (vital capacity and forced expiratory volume in 1 s), nervous system, liver enzymes or blood counts were not found. The frequency of micronuclei after mitotic stimulation with phytohaemagglutinin was higher among the deck crew (mean 4.2 SEM 0.40) than in other crew members (mean 3.6, SEM 0.35). although the difference was not statistically significant. We found no association between exposure and the frequency of sister chromatid exchanges or micronuclei after stimulation with pokeweed mitogen. CONCLUSION: This study indicates that exposure to cargo vapours in coastal tanker crews may cause symptoms in the respiratory and nervous systems.

Norppa H. **Cytogenetic markers of susceptibility: influence of polymorphic carcinogen-metabolizing enzymes.** Environ Health Perspect 1997;105(Suppl 4):829-35.
Polymorphisms of xenobiotic-metabolizing enzymes, responsible for individual differences in metabolic activation and detoxification reactions, may profoundly modulate the effects of chemical carcinogens. In the case of genotoxic carcinogens, differences in biological effects due to genetic polymorphisms can be evaluated by cytogenetic methods such as the analysis of chromosomal aberrations (CAs), sister chromatid exchanges (SCEs), micronuclei (MN), and changes in chromosome number. These techniques can be applied to any exposure known to induce such alterations, without additional method development for each exposing agent. The influence of polymorphic genes on the cytogenetic effects of a carcinogen can quickly be tested in vitro using metabolically competent cells collected from donors representing different genotypes or phenotypes. For instance, erythrocytes from individuals positive for glutathione S-transferase T1 (GSTT1) express GSTT1, whereas GSTT1-null donors, having a homozygous deletion of the GSTT1 gene, completely lack this detoxification enzyme. This deficiency results in highly increased sensitivity to SCE induction in whole-blood lymphocyte cultures by 1,2:3,4-diepoxybutane, a reactive metabolite of 1,3-butadiene. The same cytogenetic techniques can also be applied as effect biomarkers in studies of human populations exposed to genotoxic carcinogens. For example, elevated rates of chromosome damage have been detected among smokers lacking glutathione S-transferase M1 (GSTM1-null genotype), and the baseline level of SCEs seems to be increased in GSTT1-null individuals. Information obtained from cytogenetic studies of genetic polymorphisms can be used, for example, to recognize the genotoxically relevant substrates of the polymorphic enzymes, to identify genotypes that are susceptible to these genotoxins, to improve in vitro genotoxicity tests utilizing human cells, to increase the sensitivity of cytogenetic endpoints as biomarkers of genotoxic effects in humans, and to direct mechanistic studies and cancer epidemiology.

Odagiri Y, Uchida H, Shibazaki S. **Interindividual variation in cytogenetic response to X-ray and colchicine measured with the cytokinesis-block micronucleus assay.** Mutat Res 1997;381(1):1-13.
Interindividual variation in cytogenetic response to two different types of micronucleus (MN) inducer, X-rays (a clastogen) and colchicine (a spindle poison), was investigated in the peripheral blood

lymphocytes of normal healthy donors by the cytokinesis-block MN method. The data for 124 donors between the ages of 19 and 80 years showed that the histogram of individual frequency of X-ray (2 Gy)-induced micronucleated cells followed the normal distribution (Shapiro Wilks W-test) with a significant interindividual variance (ANOVA, $p < 0.001$). This was, however, not the case for colchicine (0.03 microgram/ml)-induced micronucleated cells. Instead, a skewed distribution illustrating interindividual variation was evident (ANOVA, $p < 0.001$). Statistical analysis of the effect of age and sex on MN incidence by using the Kruskal-Wallis test indicated that age affected the baseline and colchicine-induced MN incidences strongly but not the X-ray-induced MN incidence. There was no effect of sex on the incidence of micronuclei induced by either agent. In order to avoid any possible effect of age on the MN index, data for young subjects aged less than 30 years old were analyzed separately. The results of this analysis again showed significant interindividual variations in baseline, X-ray-induced, and colchicine-induced micronucleated cell rates. Results of the correlation-coefficient analysis showed that neither X-ray-induced MN incidence nor colchicine-induced MN incidence was related to baseline MN incidence. No correlation between X-ray-induced and colchicine-induced MN incidences was also found by this analysis. These results suggest that interindividual variance in chromosomal response to mutagens in normal populations may be a real phenomenon, as is interindividual variance in baseline MN frequency, and that individual susceptibilities to the two different types of micronucleus inducers (X-ray and colchicine) are unrelated, and the baseline MN level is not of predictive value for the susceptibilities.

Ogburn CE, Oshima J, Poot M, Chen R, Hunt KE, Gollahon KA, Rabinovitch PS, Martin GM. **An apoptosis-inducing genotoxin differentiates heterozygotic carriers for Werner helicase mutations from wild-type and homozygous mutants.** Hum Genet 1997;101(2):121-5.
Immortalized B lymphocytes from Werner syndrome subjects are shown to be hypersensitive to 4-nitroquinoline-1-oxide (4NQO), supporting earlier work on T lymphocytes. We also show that B cell lines from clinically normal heterozygous carriers exhibit sensitivities to this genotoxic agent, which are intermediate to those of wild-type and homozygous mutants. 4NQO is shown to induce an apoptotic response. These data encourage research on DNA repair with such cell lines and raise the question of an enhanced sensitivity of the relatively prevalent heterozygous carriers to certain environmental genotoxic agents.

Ohe T. **Quantification of mutagenic/carcinogenic heterocyclic amines, MeIQx, Trp-P-1, Trp-P-2 and PhIP, contributing highly to genotoxicity of river water.** Mutat Res 1997;393(1-2):73-9.
Four mutagenic/carcinogenic heterocyclic amines (HCAs), 2-amino-3,8-dimethylimidazo[4,5-f] quinoxaline (MeIQx), 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1), 3-amino-1-methyl-5H-pyrido[4,3-b]indole(Trp-P-2) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), in organic extracts obtained by blue rayon hanging method from the Yodo River water were quantified. Blue rayon extracts obtained were separated in two stages of fractionation by reversed-phase high performance liquid chromatography (HPLC), and the quantification of corresponding fractions was performed by HPLC with an electrochemical detector for MeIQx and a fluorometric detector for Trp-P-1, Trp-P-2 and PhIP. The geometrical mean values of MeIQx, Trp-P-1, Trp-P-2 and PhIP in extracts collected at 11 locations from the Yodo River systems were 4.8, 26.9, 37.3, and 11.9 ng/g blue rayon equivalent, respectively. The total amounts of four HCAs accounted for mean 24% of the genotoxicity of blue rayon

extracts evaluated by the umu test using an O-acetyltransferase-overproducing strain NM2009.

Okai Y, Higashi-Okai K. **Potent suppressing activity of the non-polyphenolic fraction of green tea (Camellia sinensis) against genotoxin-induced umu C gene expression in Salmonella typhimurium (TA 1535/pSK 1002) - association with pheophytins a and b.** Cancer Lett 1997;120(1):117-23.
BIOSIS COPYRIGHT: BIOL ABS. Antigenotoxic and antimutagenic activities of green tea extract and tea-derived polyphenols have been studied using in vitro and in vivo experiments. However, antigenotoxic substances in the non-polyphenolic fraction of green tea have been poorly elucidated. In the present study, the effect of the non-polyphenolic fraction of green tea on genotoxin-induced umu C gene expression was analyzed using a tester bacteria, and potent antigenotoxic substances in the non-polyphenolic fraction were identified. The non-polyphenolic fraction of green tea showed strong suppressive activities against umu C gene expression in Salmonella typhimurium (TA 1535/pSK 1002) induced by 3-amino-1,4-dimethyl-5H-pyrido(4,3-b)indol (Trp-P-1) or mitomycin C (MMC) in the presence or absence of S9 metabolizing enzyme mixture. The non-polyphenolic fraction of green tea exhibited major two-color bands in a silica gel TLC and they were identified as chlorophyll-related compounds, pheophytins a and b, judged by their specific colors, Rf values in silica gel TLC and absorption spectra. These pigments showed significant suppressive activities against umu C gene expression in tester bacteria induced by Trp-P-1 and MMC in a dose-dependent manner. These results suggest that the non-polyphenolic fraction of green tea contains pheophytins a and b as potent antigenotoxic substances.

Olive PL, Banath JP. **Multicell spheroid response to drugs predicted with the comet assay.** Cancer Res 1997;57(24):5528-33.
Multicell spheroids were exposed to DNA-damaging agents with the aim of determining whether prompt DNA damage could be predictive for cell killing and drug resistance. Chinese hamster V79 cells, SiHa human cervical carcinoma cells, and WiDr human colon carcinoma cells were grown as spheroids and exposed to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), 4-nitroquinoline-1-oxide (4NQO), doxorubicin, etoposide, actinomycin D, 1-(2-nitro-1-imidazolyl)-3-aziridino-2-propanol (RSU 1069), 3-amino-1,2,4-benzotriazine-1,4-dioxide (tirapazamine), and nitrogen mustard. Average DNA damage measured using the alkali comet assay generally correlated with cell killing irrespective of exposure times or drug concentration. However, better predictive power was achieved by using DNA damage levels in individual cells to identify the fraction of cells containing sufficient numbers of DNA strand breaks to cause death. Using this concept of a threshold for DNA damage, cell survival could be predicted for exposure to 4NQO, tirapazamine, nitrogen mustard, RSU 1069, and actinomycin D and was largely independent of cell type. The threshold value varied for each drug. For 4NQO, tirapazamine, and RSU 1069, DNA damage equivalent to about 10,000 strand breaks/cell was not toxic to cells of any spheroid type. Conversely, for actinomycin D, any DNA damage above background levels (approximately 100 breaks) was toxic for all three cell types. For some DNA-damaging drugs, the lack of correlation between DNA damage and cell killing was also informative. For etoposide and doxorubicin, no common threshold for cell killing could be determined, consistent with the hypothesis that DNA damage is only one of the actions of these drugs leading to cell death. For MNNG, the tail moment threshold varied significantly for the different spheroid types, probably indicating differences in repair. Overall, for five of the eight drugs, DNA damage measured using the comet assay was an

effective and quantitative method of predicting drug cytotoxicity in complex multicelled systems.

Panneerselvam N. **Genotoxic effects of poly-D-lysine in m-AMSA (amsacrine) treated Chinese hamster ovary (CHO) cells.** Indian J Exp Biol 1997;35(6):658-60.
Cultured Chinese hamster ovary (CHO) cells were pre-treated with m-AMSA (amsacrine) and post-treated with different doses of polycationic compound poly-D-lysine (PDL) during G2 period in order to test on the frequency of chromatid-type aberrations. The present results indicate that PDL enhances the genotoxic action of m-AMSA measured as induced chromatid aberrations.

Paul SF, Venkatachalam P, Jeevanram RK. **Analysis of radiation dose-response curve obtained with cytokinesis block micronucleus assay.** Nucl Med Biol 1997;24(5):413-6.
The frequency of micronuclei and acentrics obtained with different doses of 60Co gamma radiation was examined. When compared to acentric frequency the micronuclei frequency was found to be higher at about 115% for doses below 1 Gy. However, it dropped to about 65% as the dose was increased to 4 Gy. This paper discusses the causes for the reduced frequency of micronuclei at higher doses by taking into account the possibility of their being masked from view by the daughter nuclei in the binucleated cell.

Paz-Elizur T, Barak Y, Livneh Z. **Anti-mutagenic activity of DNA damage-binding proteins mediated by direct inhibition of translesion replication.** J Biol Chem 1997;272(46):28906-11.
DNA lesions that block replication can be bypassed in Escherichia coli by a special DNA synthesis process termed translesion replication. This process is mutagenic due to the miscoding nature of the DNA lesions. We report that the repair enzyme formamido-pyrimidine DNA glycosylase and the general DNA damage recognition protein UvrA each inhibit specifically translesion replication through an abasic site analog by purified DNA polymerases I and II, and DNA polymerase III (alpha subunit) from E. coli. In vivo experiments suggest that a similar inhibitory mechanism prevents at least 70% of the mutations caused by ultraviolet light DNA lesions in E. coli. These results suggest that DNA damage-binding proteins regulate mutagenesis by a novel mechanism that involves direct inhibition of translesion replication. This mechanism provides anti-mutagenic defense against DNA lesions that have escaped DNA repair.

Pfau W, Brockstedt U, Shirai T, Ito N, Marquardt H. **Pancreatic DNA adducts formed in vitro and in vivo by the food mutagens 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-amino-3-methyl-9H-pyrido[2,3-b]indole (MeA alphaC).** Mutat Res 1997;378(1-2):13-22.
Genotoxic heterocyclic amines have been detected in grilled or fried meat and tobacco smoke. Among these, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-amino-3-methyl-9H-pyrido[2,3-b]indole (MeA alphaC) have been shown to induce tumours in rodents in several organs. Here we report on the DNA adduct formation by PhIP and MeA alphaC in vitro and in vivo, both in rat hepatic and rat pancreatic tissues or cells. Using 32P-postlabelling analysis both compounds were shown to induce a dose-dependent DNA modification in primary rat hepatocytes that was correlated with cytotoxicity in these cells. In explanted rat pancreas maintained in dynamic short-term organ culture MeA alphaC was shown to induce covalent DNA adducts. No DNA adducts were observed with PhIP in this assay. DNA adducts were observed in the liver and the pancreas of F344 rats treated with PhIP, with a 36-times higher level of adducts in the pancreas, confirming data reported earlier. DNA adduct levels induced by

feeding 32, 160 or 800 ppm MeA alphaC in the diet were dose-dependent and higher in the liver compared with other organs including pancreas. While for PhIP the N2-(desoxyguanin-8-yl)-derivative was accounting for more than 90% of DNA adducts detected, in the case of MeA alphaC the N2-(desoxyguanin-8-yl) adduct was predominant in vitro and determined in vivo as one of up to 5 DNA adducts. MeA alphaC had been reported to induce preneoplastic foci and tumours in the liver and tumours and atrophy in the pancreas. In the case of MeA alphaC, the DNA adduct formation and cytotoxicity observed by us in vitro and in vivo correlate with the organ specificity of the reported pathological lesions. In the case of PhIP our in vitro data in pancreas and liver and the low adduct levels in liver in vivo also reflect the reported lack of pathological effects in these organs. In contrast, in pancreas, in vivo extraordinarily high adduct levels induced by PhIP were observed confirming studies published earlier, in spite of the fact that this compound does not cause pancreatic lesions. This enigmatic observation is discussed and the relevant literature is reviewed.

Pfau W, Schulze C, Shirai T, Hasegawa R, Brockstedt U. **Identification of the major hepatic DNA adduct formed by the food mutagen 2-amino-9H-pyrido[2,3-b]indole (A alpha C).** Chem Res Toxicol 1997;10(10):1192-7.
2-Amino-9H-pyrido[2,3-b]indole (A alpha C) is among the most prevalent heterocyclic amines detected in grilled or panfried meat; it was shown to be carcinogenic in mice, to induce preneoplastic foci in rat liver, and to form covalent DNA adducts in vitro and in vivo. The corresponding nitro compound 2-nitro-9H-pyrido[2,3-b]indole (N alpha C) was prepared and shown to be a direct acting mutagen in the Salmonella assay, while the amino compound required external metabolic activation with rat liver homogenate (S9). When A alpha C was incubated with S9 in the presence of calf thymus DNA, one major DNA adduct spot was detected upon 32P-postlabeling analysis. This adduct comigrated on ion-exchange TLC and reversed-phase HPLC with the major adduct detected in primary hepatocytes treated with A alpha C. In DNA isolated from livers of male F344 rats treated with 800 and 160 ppm, the formation of the same major adduct was observed with relative adduct levels of 20.6 +/- 9.6 and 1.4 +/- 1.1 adducts/10(8), respectively, as determined with the butanol extraction variant of the 32P-postlabeling assay. No DNA adducts were detected in liver DNA from rats treated with 32 ppm A alpha C or control animals. The major adduct spot was eluted and hydrolyzed and the modified base characterized by chromatographic and UV spectral comparison with a synthetic standard synthesized from acetylated guanine N3-oxide and A alpha C. Electrospray mass spectrometry and 1H- and 13C-NMR spectroscopy provided further evidence for the major adduct as N2-(guanin-8-yl)-2-amino-9H-pyrido[2,3-b]indole. A alpha C is formed especially in high-temperature preparation of food and may contribute considerably to the human carcinogenic risk that might be imposed by heterocyclic amines.

Philipose B, Singh R, Khan KA, Giri AK. **Comparative mutagenic and genotoxic effects of three propionic acid derivatives ibuprofen, ketoprofen and naproxen.** Mutat Res 1997;393(1-2):123-31.
The mutagenicity of three propionic acid derivatives, namely ibuprofen, ketoprofen and naproxen, was tested in the Ames mutagenicity assay (in strains TA97a, TA100 and TA102) and in vivo genotoxicity was tested by sister chromatid exchange (SCE) in bone marrow cells of mice. These are the anti-inflammatory drugs frequently used in different parts of the world. Mutagenicity results showed no mutagenic effects in strains TA97a, TA100 and TA102 for all three drugs. Results of in vivo SCE assays indicate that these three drugs are weakly genoxic in bone marrow cells of mice. This is the first report

of the Ames mutagenicity assay for ketoprofen and in vivo SCE assay for three drugs.

Picada JN, Silva KV, Erdtmann B, Henriques AT, Henriques J A. **Genotoxic effects of structurally related beta-carboline alkaloids.** Mutat Res 1997;379(2):135-49.
BIOSIS COPYRIGHT: BIOL ABS. beta-Carboline alkaloids, found in medicinal plants, tobacco smoke and well-cooked foods, have shown a variety of actions in biological systems related to their interaction with DNA. Therefore, these alkaloids can be considered potentially mutagenic. In this work, the genotoxic, mutagenic, and cytotoxic activities of three aromatic beta-carboline alkaloids (harman, harmine, and harmol) and two dihydro-beta-carboline alkaloids (harmaline and harmalol) were evaluated by means of the Salmonella/microsome assay (Salmonella typhimurium TA98, TA97, TA100, and TA102) and SOS chromotest (Escherichia coli PQ37) with and without metabolic activation. Moreover, harman and harmine were analyzed by the micronucleus assay in vivo. It was shown that genotoxicity was inhibited by the addition of S9 mix for aromatic beta-carbolines barman and harmol in TA97. However, harmine showed signs of mutagenicity only in the presence of S9 mix in TA98 and TA97 frameshift strains. In the SOS chromotest, only harman induced SOS functions in the absence of S9 mix. Dihydro-beta-carbolines were not genotoxic in any of the microorganisms used. The negative responses obtained in the micronucleus assay indicated that harman and harmine were not able to induce chromosomal mutations.

Plewa MJ, Wagner ED, Yu TW, Anderson D. **Paraoxon-mediated antimutagenicity and mutagenic synergy of dietary arylamines assayed in Salmonella typhimurium and human lymphocytes.** J Environ Pathol Toxicol Oncol 1997;16(4):303-11. BIOSIS COPYRIGHT: BIOL ABS. There is nearly universal exposure to organophosphorus ester insecticides and aromatic amines. Previously we reported that specific aromatic amines and paraoxon induced enhanced mutagenic responses in several S. typhimurium tester strains. In this study we demonstrated that paraoxon could alter the genotoxic potency of mammalian activated m-phenylenediamine (mPDA) and the dietary carcinogens 2-amino-3-methylimidazo-(4,5-f)quinoline (IQ) and 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP) as measured in S. typhimurium and in human lymphocytes. Paraoxon alone was not genotoxic in any of the assays. All three aromatic amines exhibited paraoxon-mediated mutagenic synergy when assayed with S. typhimurium strain YG1024. However, using the single cell gel electrophoresis (Comet) assay with lymphocytes, a paraoxon concentration-dependent genotoxic synergy was induced with mPDA. A bimodal response was observed with lymphocytes treated with a constant amount of IQ or PhIP and with 1 to 500 muM paraoxon. At low paraoxon concentrations a significant increase in the comet tail moment value was induced when compared with the cells treated with IQ or PhIP alone. At higher paraoxon concentrations a significant antigenotoxic response was observed. These data demonstrate that an environmental agent such as paraoxon, can induce both genotoxic synergy and antigenotoxicity in human cells depending on its concentration. This result raises the concern of the environmental effects of organophosphorus ester insecticides under real-world conditions where people are exposed to a multitude of genotoxic agents.

Poirier MC. **DNA adducts as exposure biomarkers and indicators of cancer risk.** Environ Health Perspect 1997;105(Suppl 4):907-12.
Quantitation of DNA adducts in human tissues has been achieved with highly sensitive techniques based

on adduct radiolabeling, antisera specific for DNA adducts or modified DNA, and/or adduct structural characterization using chemical instrumentation. Combinations of these approaches now promise to elucidate specific adduct structures and provide detection limits in the range of 1 adduct/10(9) nucleotides. Documentation of human exposure and biologically effective dose (i.e., chemical bound to DNA) has been achieved for a wide variety of chemical carcinogens, including polycyclic aromatic hydrocarbons (PAHs), aromatic amines, heterocyclic amines, aflatoxins, nitrosamines, cancer chemotherapeutic agents, styrene, and malondialdehyde. Due to difficulties in exposure documentation, dosimetry has not been precise with most environmental and occupational exposures, even though increases in human blood cell DNA adduct levels may correlate approximately with dose. Perhaps more significant are observations that lowering exposure results in decreasing DNA adduct levels. DNA adduct dosimetry for environmental agents has been achieved with dietary contaminants. For example, blood cell polycyclic aromatic hydrocarbon-DNA adduct levels were shown to correlate with frequency of charbroiled meat consumption in California firefighters. In addition, in China urinary excretion of the aflatoxin B1-N7-guanine (AFB1-N7-G) adduct was shown to increase linearly with the aflatoxin content of ingested food. Assessment of DNA adduct formation as an indicator of human cancer risk requires a prospective nested case-control study design. This has been achieved in one investigation of hepatocellular carcinoma and urinary aflatoxin adducts using subjects followed by a Shanghai liver cancer registry. Individuals who excreted the AFB1-N7-G adduct had a 9.1-fold adjusted increased relative risk of hepatocellular carcinoma compared to individuals with no adducts. Future advances in this field will be dependent on chemical characterization of specific DNA adducts formed in human tissues, more-precise molecular dosimetry, efforts to correlate DNA adducts with cancer risk, and elucidation of opportunities to reduce human DNA adduct levels.

Prasanna PG, Kolanko CJ, Gerstenberg HM, Blakely WF. **Premature chromosome condensation assay for biodosimetry: studies with fission-neutrons**. Health Phys 1997;72(4):594-600.
The relationship between neutron doses and excess premature chromosome condensation (PCC) fragments in human lymphocytes was studied, and a comparison was made with X-ray dose/response data for the determination of relative biological effectiveness (RBE). Lymphocytes from healthy human donors were used in X-ray and neutron irradiation dose/response experiments. Lymphocytes were isolated from whole peripheral blood on a density gradient, and suspended in a tissue culture medium at a concentration of approximately 1.5X10(6)/milliliter for irradiation. For each radiation type, unexposed cells were included as controls. Cells were placed on a rotating Plexiglas holder for irradiation and exposed at room temperature at a dose rate of 1.0 gray (Gy) per minute. X-Ray doses ranged from 0.5 to 9.0Gy. Neutron irradiation doses ranged from 0.25 to 4.0Gy at a dose rate of 0.25Gy/minute. Paired ion chambers placed at the center of the sample array were used to determine separately the doses due to gamma and neutron irradiation. Mitotic Chinese-hamster-ovary (CHO) cells were used as inducers of chromosome condensation in the prematurely condensed chromosome (PCC) assay following radiation exposures. PCC and fragment yield data were analyzed using the Papworth test. Frequency distributions of PCCs and fragments following 4.5Gy X-ray and 2.0Gy neutrons illustrated approximately equivalent yields of excess PCC fragments. PCC and fragment distribution were non-Poisson following exposure to either X-ray or neutron radiation exposure, indicating overdispersion. The number of PCCs and fragments after 24 hours of repair progressively increased following exposure to radiation with doses of X-rays and neutrons. The neutron RBE value was estimated at 2.4.

Provost GS, Mirsalis JC, Rogers BJ, Short JM. **Mutagenic response to benzene and tris(2,3-dibromopropyl)-phosphate in the lambda lacI transgenic mouse mutation assay: a standardized approach to in vivo mutation analysis**. Environ Mol Mutagen 1996;28(4):342-7.
The mutagenic activity of benzene (71432) and tris(2,3-dibromopropyl)phosphate (126727) (TDBP) was evaluated in the lambda lacI transgenic mouse mutation assay. Male B63F1-transgenic-lambda/lacI-mice were gavaged with 200, 400, or 750mg/kg benzene daily for 5 days, or 150mg/kg TDBP daily for 2 days, or 300 or 600mg/kg TDBP daily for 4 days. The mice were killed 19 days after the last injection and the lungs, femoral bone marrow, spleen, liver, kidneys, and stomach were removed. The lung, bone marrow, and spleen tissues from benzene treated mice and liver, kidney, and stomach tissues from TDBP treated mice were examined for lacI mutations in the lambda/lacI transgene. The 200mg/kg dose of benzene induced a significant increase in lacI mutation frequency in the bone marrow. The 400mg/kg dose caused a significant increase in lacI mutation frequency in the spleen. The other doses did not significantly alter the lacI mutation frequency in the bone marrow or spleen. None of the benzene doses significantly increased the lacI mutation frequency in the lungs. The 300 and 600mg/kg doses of TDBP significantly increased the lacI mutation frequency in the kidneys, but not in the liver or stomach. The 150mg/kg dose did not significantly affect the lacI frequency in any tissue. The authors conclude that the mutation pattern induced by benzene and TDBP in this study is similar to that seen in previous studies. The results of this and other studies establish the validity of using transgenic rodents as an effective relative short term assay for evaluating the genotoxic effects of tissue specific and multiple site mutagens and carcinogens in-vivo.

Ptitsyn LR, Horneck G, Komova O, Kozubek S, Krasavin EA, Bonev M, Rettberg P. **A biosensor for environmental genotoxin screening based on an SOS lux assay in recombinant Escherichia coli cells.** Appl Environ Microbiol 1997;63(11):4377-84.
A genetically controlled luminescent bacterial reporter assay, the SOS lux test, was developed for rapid detection of environmental genotoxins. The bioassay is based on the recombinant plasmid pPLS-1, which was constructed as a derivative of pBR322, carrying the promoterless luxCDABFE genes of Photobacterium leiognathi downstream of a truncated cda gene from ColD with a strong SOS promoter. E. coli recA+ strains containing this construction are inducible to high levels of light production in the presence of substances or agents that cause damage to the DNA of the cells. The light signal, reflecting the SOS-inducing potency, is recorded from the growing culture within 1 s, and the test results are available within 1 to 2 h. Induction of bioluminescence was demonstrated by treatment of E. coli C600 (pPLS-1) with 6 genotoxic chemicals (mitomycin C, N-methyl-N'-nitro-N-nitrosoguanidine, nalidixic acid, dimethylsulfate, hydrogen peroxide, and formaldehyde) and with UV and gamma radiation. A clear dose-response relationship was established for all eight genotoxins. The sensitivity of the SOS lux test is similar to that of other bioassays for genotoxicity or mutagenicity, such as the SOS chromotest, umu test, and Ames mutatest. These results indicate that the SOS lux test is potentially useful for the in situ and continuous detection of genotoxins.

Rachidi M, Lopes C, Benichou J. **Genetical analysis of visual system disorganizer (vid), a new gene involved in normal development of eye and optic lobe of the brain in Drosophila melanogaster**. Genetica 1997;99(1):31-45.

CBAC COPYRIGHT: CHEM ABS A neuroanatomical screening of a collection of P-element mutagenized flies has been carried out with the aim of finding new mutants affecting the optic lobe of the adult brain in Drosophila melanogaster. We have identified a new gene that is involved in the development of the adult axon array in the optic ganglia and in the ommatidia assembly. We have named this locus visual system disorganizer (vid). Reversional mutagenesis demonstrated that the vid mutant was the result of a P-element insertion in the Drosophila genome and allowed us to generate independent alleles, some of which resulted in semilethality, like the vid original mutant, while the others were completely lethal. A genetic somatic mosaic anal. indicated that the vid gene is required in the eye for its normal development by inductive effects. This anal. also suggests an inductive effect of the vid gene on the distal portion of the optic lobe, particularly the lamina and the first optic chiasma. Moreover, the absence of mutant phenotype in the proximal region of the optic ganglia, including the medulla, the second optic chiasma, and the lobula complex underlying mosaic eyes, is suggestive of an autonomously acting mechanism of the vid gene in the optic lobe. The complete or partial lethality generated by different mutations at the vid locus suggests that this gene's role may not be limited to the visual system, but may also affect a vital function during Drosophila development.

Reddy MV, Blackburn GR, Schreiner CA, Mackerer CR. **Correlation of mutagenic potencies of various petroleum oils and oil coal tar mixtures with DNA adduct levels in vitro.** Mutat Res 1997;378(1-2):89-95.
An in vitro system was utilized to measure DNA adduct-forming ability of petroleum oils and oil coal tar mixtures to define correlations between DNA adduct levels and their mutagenic potencies. The system consisted of reaction of dimethyl sulfoxide extracts of oils with calf thymus DNA in the presence of Aroclor-induced hamster liver microsomes for 30 min. Following DNA extraction, DNA adducts were measured by the nuclease P1-enhanced postlabeling assay coupled with two-dimensional polyethyleneimine (PEI)-cellulose TLC. Thin layer plates showed putative aromatic DNA adducts, with levels ranging from 60 to 1400 adducts per 10(9) DNA nucleotides. TLC mobilities suggested adducts to be aromatic compounds containing 4 or more rings. A good correlation (coefficient of correlation = 0.91) was observed between DNA adduct levels and Salmonella mutagenicity for 19 oils. All 19 samples tested produced DNA adducts. To expedite the TLC procedure, adducts were resolved by one-dimensional TLC and the radioactivity measured using a mechanical scanner. Results were comparable to those obtained by two-dimensional TLC and quantification after scraping. Our data show that the in vitro incubation system coupled with the postlabeling adduct assay is a useful screening method to identify mutagenic and potentially carcinogenic oils.

Ribas G, Surralles J, Carbonell E, Xamena N, Creus A, Marcos R. **Genotoxic evaluation of the herbicide trifluralin on human lymphocytes exposed in vitro**. Mutat Res 1996;371(1-2):15-21.
The genotoxicity of trifluralin (1582098) was examined in human lymphocytes. Lymphocyte cultures were established from blood samples drawn from two healthy young male donors. These were treated with 0 to 200 micrograms per milliliter (microg/ml) trifluralin with or without metabolic activation from S9 mix for up to 72 hours (hr). Induction of sister chromatid exchanges (SCEs) was assessed after 2 or 48hr of incubation with trifluralin. Micronuclei induction was evaluated after 72hr of trifluralin treatment. Induction of chromosome aberrations was assessed after 30hr of incubation with trifluralin. Cytotoxicity was assessed by measuring changes in the proliferative rate index (PRI), determined by

examination of the first three metaphases, and the cytokinesis block proliferative index (CBPI). Trifluralin treatment for 48hr in the absence of S9 mix caused a slight, but statistically significant increase in SCE frequency in lymphocytes from both donors at 50microg/ml, the highest concentration tested. Treatment with 25microg/ml trifluralin in the absence of S9 mix also caused a significant increase in SCE frequency in lymphocytes from one donor. Treatment with 200microg/ml trifluralin for 2hr in the presence of S9 mix caused a significant increase in SCE frequency in lymphocytes from both donors. These effects were accompanied by slight decreases in the PRI and CBPI. Trifluralin did not increase the frequency of chromosome aberrations or micronuclei above the background level. The authors conclude that trifluralin is able to exert weak cytotoxic and genotoxic effects in cultured human lymphocytes. The SCE assay seems to be more sensitive for detecting these types of effects than the chromosome aberration or micronucleus assays.

Rosser PF, Ramachandran P, Sangaiah R, Austin RN, Gold A, Ball LM. **Role of O-acetyltransferase in activation of oxidised metabolites of the genotoxic environmental pollutant 1-nitropyrene**. Mutat Res 1996,369(3-4):209-20.
The importance of O-acetyltransferase (OAT) activity in the bacterial mutagenicity of nitrated polycyclic aromatic hydrocarbons (NPAH) was evaluated in the Ames Salmonella typhimurium assay. Oxidized metabolites of 1-nitropyrene (5522430) were tested at doses ranging from 0.005 to 5 micrograms per plate, with and without metabolic activation. The nitropyrenols showed some mutagenicity without activation. Involvement of OAT was indicated by decreased activity in strain TA-98/1,8DNP6, compared with TA-98 and enhanced potency in YG1024. The presence of metabolic activation enhanced the potency of all the nitropyrenols in TA-98. 1-Nitropyren-4-ol (91254965) proved more potent than the nitropyrenols tested previously and showed substantial activity in the absence of activation. 1-Nitropyren-6-ol (1767288) was most sensitive to loss of activity in the acetylase deficient TA-98/1,8DNP6 variant, and was also strongly enhanced in YG1024 with or without activation. Acetamide derivatives were active with activation and were enhanced about three fold in strain YG1024. In the absence of OAT, these products were inactive with or without activation. The authors conclude that the importance of esterification in generating active species from NPAH emphasizes the role played by the metabolic specificity of individual tissues in determining susceptibility to genotoxicity of NPAH.

Sanyal R, Darroudi F, Parzefall W, Nagao M, Knasmuller S. **Inhibition of the genotoxic effects of heterocyclic amines in human derived hepatoma cells by dietary bioantimutagens.** Mutagenesis 1997;12(4):297-303.
The effects of dietary bioantimutagens (compounds which have been shown to inhibit mutagenesis via interaction with DNA repair processes) on spontaneous and heterocyclic amine (HCA)-induced micronucleus (MN) frequencies were studied in metabolically competent human hepatoma (Hep-G2) cells. All the compounds tested (coumarin, vanillin, caffeine, tannic acid and cinnamaldehyde) caused a moderate increase of MN numbers in Hep-G2 cells at high concentrations (500 microg/ml); only tannic acid was also active at lower dose levels. In combination experiments with the HCA 2-amino-3-methylimidazo-[3,4-f]quinoline (IQ), post-treatment of the cells with bioantimutagens resulted in a pronounced (75-90%) decrease in MN. The most drastic effects were seen with vanillin, coumarin and caffeine which were active at concentrations < or = 5 microg/ml. Further experiments indicated that these compounds also attenuate the mutagenic effects of other HCAs (PhIP, MeIQ, MeIQx, Trp-P-1).

Sarrif AM, Krahn DF, Donovan SM, O'Neil RM. **Evaluation of hexamethylphosphoramide for gene mutations in Salmonella typhimurium using plate incorporation, preincubation, and suspension assays.** Mutat Res 1997;380(1-2):167-77.
Hexamethylphosphoramide (HMPA), a potent rat nasal carcinogen by inhalation, and three of its metabolites, pentamethylphosphoramide (PMPA), trimethylphosphoramide (TriMPA), and formaldehyde (HCHO), were assessed in Salmonella typhimurium gene mutation assays using various protocols, including plate incorporation, preincubation and suspension assays. HMPA (tested up to 15,000 micrograms/plate) was not mutagenic in plate incorporation or preincubation assays with or without metabolic activation. HCHO was mutagenic in the plate incorporation and preincubation assays (tested up to 150 micrograms/plate). In suspension assays, however, HMPA (tested up to 40 mg/ml), PMPA (up to 44 mg/ml) and HCHO (up to 45 micrograms/ml), but not TriMPA (up to 29 mg/ml), were mutagenic. HMPA and PMPA were positive only with activation. HMPA's mutagenicity was optimized using a relatively high level of rat liver S9 protein (3.5 mg/plate) in the metabolic activation mixture. Semicarbazide, an HCHO trapping agent, added at concentrations up to 167 micrograms/ml, markedly inhibited the mutagenic activities of HMPA and PMPA suggesting that HCHO generation may play a role in their mutagenicity. These studies show that HMPA is mutagenic in a modified Salmonella typhimurium reverse mutation assay with metabolic activation. Successive N-demethylation of HMPA eventually eliminates the mutagenic activity which further suggests that HMPA's mutagenic activity is related to the release of HCHO.

Sasaki JC, Arey J, Eastmond DA, Parks KK, Grosovsky AJ. **Genotoxicity induced in human lymphoblasts by atmospheric reaction products of naphthalene and phenanthrene.** Mutat Res 1997;393(1-2):23-35.
BIOSIS COPYRIGHT: BIOL ABS. The genotoxic risks from exposure to polycyclic aromatic hydrocarbons (PAH) have long been recognized. Less well understood are the potential genotoxic risks of the atmospheric reaction products of this class of compounds. In this investigation, we have utilized several human cell genotoxicity assays to evaluate naphthalene, phenanthrene, and their atmospheric reaction products 1-nitronaphthalene, 2-nitronaphthalene, 1-hydroxy-2-nitronaphthalene, 2-hydroxy-1-nitronaphthalene, 1,4-naphthoquinone and 2-nitrodibenzopyranone. In addition, reaction products of naphthalene were generated in a 6700-l Teflon environmental chamber, collected on a solid adsorbent, extracted and fractionated by normal-phase HPLC. Individual fractions were then analyzed using GC-MS, and tested for genotoxicity. Genotoxicity was determined using the human B-lymphoblastoid cell line, MCL-5, which expresses several transfected P450 and epoxide hydrolase genes. Mutagenicity was evaluated at both the heterozygous tk locus and the hemizygous hprt locus, permitting detection of both intragenic and chromosomal scale mutational events. Test compounds were also screened using the CREST modified micronucleus assay. Genotoxicity results indicate that 2-nitronaphthalene and 2-nitrodibenzopyranone possess greater mutagenic potency than their parent compounds, and interestingly, both compounds induced significant increases in mutation frequency at tk but not hprt. These results suggest a mechanistic difference in human cell response as compared to bacteria, where both compounds were previously shown to induce point mutations in the Salmonella reversion assay. The genotoxicity of 2-nitroaphthalene and 2-nitrodibenzopyranone in human cells, together with their high concentrations in ambient air relative to nitro-PAH directly emitted from combustion sources, emphasizes the need to

consider atmospheric reaction products of PAH in genotoxicity assessments.

Sasaki YF, Izumiyama F, Nishidate E, Ishibashi S, Tsuda S, Matsusaka N, Asano N, Saotome K, Sofuni T, Hayashi M. **Detection of genotoxicity of polluted sea water using shellfish and the alkaline single-cell gel electrophoresis (SCE) assay: a preliminary study.** Mutat Res 1997;393(1-2):133-9.
We exposed two species of shellfish, Patunopecten yessoensis and Tapes japonica, for 4 h to artificial sea water in which N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), ethyl nitrosourea (EMS), 3-chloro-4-dichloromethyl-5-hydroxy-2(H)-furanone (MX), or benzo[a]pyrene (B[a]P) were dissolved. We then assessed the DNA damage in cells isolated from the gills using the alkaline single-cell gel electrophoresis (SCG) assay. A statistically significant increase in DNA damage was observed for all exposures. Therefore, the alkaline SCG assay detected DNA damage in gill cells produced by direct mutagens and promutagen dissolved in sea water. T. japonica was exposed to sea water sampled from two Pacific Ocean coasts of Japanese local cities--Hachinohe (Aomori Prefecture, Tohoku) and Nakatsu (Oita Prefecture, Kyushu)--and three bay coasts of the industrial megalopolises--Tokyo, Osaka, and Kobe. A significant increase in DNA damage was observed after the exposure to sea water from Tokyo, Osaka, and Kobe, but not from Hachinohe and Nakatsu. These results suggested the utility of the alkaline SCG assay with shellfish gill cells for monitoring sea water genotoxicity.

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.
Recently, we designed a fast and simple method to obtain nuclei for the alkaline SCG assay and we tested it with mouse liver, lung, kidney, spleen, and bone marrow. Instead of isolating organ cells by trypsinization, we homogenized tissue and isolated the nuclei. Each organ was minced, and the mince was suspended in chilled homogenizing buffer containing NaCl and Na2EDTA, homogenized gently using a Potter-type homogenizer set in ice, and then centrifuged. The nuclei from the precipitate were used for the assay. To evaluate the validity of this method, we tested the genotoxicity in mouse organs of 11 chemical 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 aromatic amines (2-AAF and phenacetin), a polycyclic aromatic hydrocarbon (B[a]P), and two inorganic chemicals (KBrO3 and K2CrO4) increased migration of the DNA from mouse organs. 5-FU (a base analog) and colchicine (a spindle poison) treatment produced negative results in all organ studied. Considering that the alkaline 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 chemical mutagens as a function of their modes of action.

Sasaki YF, Nishidate E, Izumiyama F, Watanabe-Akanuma M, Kinae N, Matsusaka N, Tsuda S. **Detection of in vivo genotoxicity of 3-chloro-4-(dichloromethyl)-5-hydroxy-2[5H]-furanone (MX) by the alkaline single cell gel electrophoresis (Comet) assay in multiple mouse organs.** Mutat Res 1997;393(1-2):47-53.
We tested the genotoxicity of 3-chloro-4-(dichloromethyl)-5-hydroxy-2[5H]-furanone (MX) in the mouse in 6 organs (liver, lung, kidney, brain, spleen, and bone marrow) and in the mucosa of stomach,

jejunum, ileum, colon, and bladder using the alkaline single-cell gel electrophoresis (SCG) (Comet) assay modified by us. Mice were sacrificed 1, 3, 6, and 24 h after oral administration of the mutagen at 100 mg/kg. MX yielded statistically significant DNA damage in the liver, kidney, lung, and brain and in all the mucosa samples. While DNA damage persisted in the gastrointestinal and urinary tract for 6-24 h after a single oral dosing, it peaked in the liver at 1 h and returned to almost the control level at 3 h. Our present results suggest that MX is genotoxic for various mouse organs, but not for the hematopoietic system, and that the alkaline SCG assay with a homogenization technique can be used to predict genotoxicity in the gastrointestinal and urinary tracts.

Schweikl H, Schmalz G. **Glutaraldehyde-containing dentin bonding agents are mutagens in mammalian cells in vitro.** J Biochem Mater Res 1997;36(3):284-8.
BIOSIS COPYRIGHT: BIOL ABS. The mutagenic potential of glutaraldehyde-containing dentin bonding agents was shown in previous studies using a bacterial gene mutation assay, the Ames test. However, current strategies of genotoxicity testing and regulatory requirements for the biological evaluation of medical devices recommend a battery of tests that indicate induced mutations in prokaryotic and eukaryotic cells. Accordingly, the mutagenicity of three glutaraldehyde-containing bonding agents (Syntac adhesive, Prisma Universal Bond 3 adhesive, and Gluma 3) was investigated using a quantitative mammalian cell gene mutation assay (V79/HPRT test) in the present investigation. The materials were extracted in dimethyl sulfoxide (0.1 g/2 mL) for 24 h and original extracts were then serially diluted in cell culture medium before exposure to V79 cells. Cytotoxic and mutagenic effects were observed with identical concentrations of extracts of the different test materials. There was a moderate decrease of the number of surviving cells immediately after the end of exposure. Mutagenicity at the hprt locus in V79 cells was found with all materials tested, and the increases in the absolute numbers of mutants were dose dependent. The mutant frequencies were about 15- (Syntac adhesive and Gluma 3) to 20-fold (Prisma UB3 adhesive) higher than solvent control values. Since other substances than glutaraldehyde may be responsible for the mutagenic effects in mammalian cells in this study, work is currently in progress to identify the individual mutagenic compounds of dentin adhesives and related composite materials.

Semov AB, Ptitsina SN, Semova NY. **[Specificity of DNA repair under chronic influence of mutagenic factors]**. Radiats Biol Radioecol 1997;37(4):565-8. (Rus)
BIOSIS COPYRIGHT: BIOL ABS. Long-time in vivo influence of chemical mutagens in low doses can decrease the level of unscheduled DNA synthesis (UDS) induced in human as well as in laboratory mammals. The phenomenon under investigation is not specific neither for chronically acting mutagens nor for challenging agent. A decrease in UV- and gamma-ray-induced UDS was registered after chronic irradiation in plant populations and also in Chernobyl ameliorators and inhabitants of radioactively contaminated regions. The observed effect seems to have general biological character.

Shukla R, Liu T, Geacintov NE, Loechler EL. **The major, N2-dG adduct of (+)-anti-B[a]PDE shows a dramatically different mutagenic specificity (predominantly, G --> A) in a 5'-CGT-3' sequence context.** Biochemistry 1997;36(33):10256-61.
Mutations induced by the (+)-anti diol epoxide of benzo[a]pyrene [(+)-anti-B[a]PDE] were described previously in the supF gene of the Escherichia coli plasmid pUB3 [Rodriguez et al.(1993) Biochemistry,

32, 1759]. (+)-anti-B[a]PDE induced a complex pattern of mutations, including insertions, deletions, frameshifts, as well as base substitution mutations, which for G:C base pairs alone included a significant fraction of G:C --> T:A, A:T and C:G mutations. A variety of results suggest that most of these mutations arise from the major adduct ([+ta]-B[a]P-N2-dG), raising the question how can a single adduct induce different kinds of mutations? Our working hypothesis in this regard is that (1) an adduct can adopt multiple conformations; (2) different conformations cause different mutations; and (3) adduct conformation is controlled by various factors, such as DNA sequence context. To investigate what conformation is associated with what mutation, it is essential to find examples where [+ta]-B[a]P-N2-dG induces principally one kind of mutation as a prelude to the study in that same context of the conformation(s) potentially relevant to mutagenesis. Earlier work indicated that (+)-anti-B[a]PDE gave a preponderance of G --> A mutations in a 5'-CGT-3 sequence context, and herein it is shown that these mutations are likely to be attributable to the major adduct, since in this same sequence context [+ta]-B[a] P-N2-dG studied site specifically also induces principally G --> A mutations (approximately 82%). Previously, [+ta]-B[a]P-N2-dG was shown to induce principally G --> T mutations (approximately 97%) in a 5'-TGC-3' sequence context. Thus, by simply altering its surrounding sequence context this adduct can give a preponderance of either G --> A or G --> T mutations. This is the most dramatic change in base substitution mutagenic specificity for an adduct described to date and illustrates that the qualitative pattern of mutagenesis by a bulky adduct can be remarkably diverse.

Skopek TR, Kort KL, Marino DR, Mittal LV, Umbenhauer DR, Laws GM, Adams SP. **Mutagenic response of the endogenous hprt gene and lacI transgene in benzo(a)pyrene-treated Big Blue B6C3F1 mice**. Environ Mol Mutagen 1996;28(4):376-84.996 The mutagenic responses of the hprt gene and lacI transgene to benzo(a)pyrene (50328) (BaP) were studied in Big-Blue-mice (BB). Male BB-mice and B6C3F1-mice were injected every other day with one to three doses of 50mg/kg BaP to give cumulative doses of 50, 100, or 150mg/kg BaP. Three weeks after the last dose the mice were killed and the spleens were scored for 6-thioguanine resistant mutations at the hprt locus. Splenic tissue from BB-mice was analyzed for lacI mutations in the lacI transgene. Additional BB and B6C3F1-mice were injected with 50mg/kg BaP. They were killed 24 hours later and the liver, spleens, and lungs were removed. The DNA was extracted from the tissues and analyzed for BaP/DNA adducts by the phosphorus-32 post labeling technique. Cytochrome-P-450 isoforms in the tissues were examined by Western blotting and enzyme linked immunosorbent assay techniques. BaP induced dose related increases in hprt mutation frequency in both species. Induction of these mutations in BB-mice, however, was two to six fold lower than in B6C3F1-mice. The single 50mg/kg BaP dose induced BaP/DNA adducts in all tissues. The level of DNA adduction in BB-mice was 30 to 50% lower in BB-mice than in B6C3F1-mice. The reduced level of DNA adduction in BB-mice correlated with the decreased hprt response. The level of 1A2, 2B, 3A, and 4A isoforms of P450 in each tissue were similar in the two mouse strains. The mean and median concentrations of cytochrome-P4501A1 in the liver was significantly lower in BB-mice than in B6C3F1-mice. The liver P4501A1 concentration correlated with the level of DNA adduction in the liver, lung, and spleens. BaP induced lacI mutations to a far greater extent, 15 to 40 fold higher, than hprt mutations. The authors conclude that in BB-mice BaP induces a much higher level of mutations in the lacI gene than hprt mutations.

Slamenova D, Collins A, Wsolova L, Papsova E, Gabelova A, Dusinska M. **DNA-synthesis inhibition**

and repair DNA-synthesis in CHO Ade- C cells: an alternative approach to genotoxicity testing. Neoplasma 1997;44(4):247-52.
BIOSIS COPYRIGHT: BIOL ABS. We describe an alternative assay to determine genotoxicity. Its main feature is that it combines two measures in a single experiment; the inhibition of replicative DNA synthesis together with the stimulation of DNA repair. We show that, in tests of four different genotoxic agents, the assay gives results that are entirely consistent with what is known about the mode of action of these agents. In addition, we have demonstrated that chemical carcinogens requiring metabolic activation can be examined using a standard procedure of incubation with a microsomal activating fraction. We consider the combined assay for DNA synthesis inhibition and repair synthesis to be a useful way for the rapid prescreening of chemicals suspected of genotoxic activity on the level of mammalian cells.

Spivak IM, Pleskach NM, Mikhel'son VM, Bootsma D, Kolman A. **[Decreased survivability and a DNA repair defect in the cells of patients with xeroderma pigmentosum and Cockayne syndrome under the action of radiation and chemical mutagens]**. Tsitologiia 1997;39(6):420-34. (Rus)
The action of ionizing radiation and chemical mutagens--epoxides (ethylene oxide, propylene oxide, epichlorohydrin)--upon survival and repair processes in xeroderma pigmentosum (XP2SP) and Cockayne syndrome (CS1SP) patients' cells was studied, compared to healthy donor's cells VH-10 and C5RO. Ionizing radiation was demonstrated to enhance significantly higher survival decrease of XP2SP and CS1SP fibroblasts, compared to healthy donor's cells, according to the cloning efficiency criterion. In contrast to this, no significant difference between XP2SP and healthy donor's cells was found, according to cells' ability to replicative DNA synthesis after gamma irradiation. Differences in survival of mutant cells and healthy donor's cells after treatment by epoxides were found significant only following XP2SP being treated by ethylene oxide. DNA single-string breaks in XP2SP and in CS1SP cells treated by mutagens studied were proved to occur with the same frequency as in the DNA of the control cells; however the DNA repair according to this criterion was significantly suppressed in mutant cells.

Suzuki M, Matsui K, Yamada M, Kasai H, Sofuni T, Nohmi T. **Construction of mutants of Salmonella typhimurium deficient in 8-hydroxyguanine DNA glycosylase and their sensitivities to oxidative mutagens and nitro compounds.** Mutat Res 1997;393(3):233-46.
8-Hydroxyguanine (8-OH-G) DNA glycosylase is an enzyme involved in repair of oxidative DNA damage, e.g., 8-OH-G in DNA. In order to assess the roles of 8-OH-G in spontaneous and chemically-induced mutagenesis, the mutMST gene encoding 8-OH-G DNA glycosylase of Salmonella typhimurium was disrupted in several Ames tester strains, i.e., S. typhimurium TA1535 (hisG46, uvrB-, rfa), TA1975 (hisG46, uvr+, rfa) and TA102 (hisG428, uvr+, rfa). The spontaneous mutation frequencies were increased 2.4 and 1.6 times, respectively, by the mutMST deletions in strains TA1535 and TA1975, which are spontaneously reverted to His+ by mutations mainly at G:C base pairs. The resulting strains YG3001 (TA1535 delta mutMST) and YG3002 (TA1975 delta mutMST) were 2 to 8 times more sensitive to the mutagenicities of methylene blue plus visible light, neutral red plus visible light and 2-nitrofluorene than the parent strains. The strain YG3002 but not YG3001 was about 30 times more sensitive to the mutagenicity of 4-nitroquinoline N-oxide than the parent strain TA1975. Neither hydrogen peroxide nor phenazine methosulfate was mutagenic in the mutMST-deletion strains as well as

in the parent strains. In contrast, the mutMST deletion did not affect the spontaneous mutation frequency of strain TA102, which has an A:T base pair at the critical site for reversion. The sensitivities of strain TA102 to the chemicals were not enhanced by the mutMST deletion except for hydrogen peroxide. These results suggest that 8-OH-G in DNA plays important roles in spontaneous mutagenesis occurring at G:C base pairs in S. typhimurium, and some nitro aromatics such as 4-nitroquinoline N-oxide or 2-nitrofluorene as well as the photosensitizers plus visible light can produce 8-OH-G in DNA, thereby inducing mutations. In the case of 4-nitroquinoline N-oxide, 8-OH-G rather than DNA adducts seems to play major roles in mutagenesis in uvr+ background. The new strains could be useful for the evaluation of the roles of 8-OH-G in mutagenesis in S. typhimurium and permit the efficient detection of some oxidative mutagens in the environment.

Takeuchi T, Matsugo S, Morimoto K. **Mutagenicity of oxidative DNA damage in Chinese hamster V79 cells.** Carcinogenesis 1997;18(11):2051-5.
We have investigated the mutagenicity of oxidative DNA damage induced in V79 Chinese hamster lung fibroblast, and measured 8-hydroxydeoxyguanosine (8OHdG) levels as an indicator of this damage. A hydroxyl radical generator, N,N'-bis(2-hydroxyperoxy-2-methoxyethyl)-1,4,5,8-naphthalene-tetr- a -carboxylic-diimide (NP-III), induced 8OHdG in V79 upon irradiation with 366 nm ultraviolet light (UV) for 15 min. 8OHdG was determined by HPLC with electrochemical detection after anaerobic sample processing. The 8OHdG level in the cells treated without NP-III was 0.49 per 10(5) dG, whereas levels in the cells treated with 5, 10 or 20 microM NP-III and UV irradiation were 1.84, 4.06 or 6.95 per 10(5) dG, respectively. The 8OHdG induced by 20 microM NP-III with UV irradiation decreased rapidly, and the half-life of the induced 8OHdG was approximately 6 h. NP-III with UV irradiation also induced DNA strand breaks in all cells uniformly, as determined by single cell gel assay. Mutant frequencies at the hypoxanthine-guanine phosphoribosyltransferase (hprt) locus in V79 were determined as the number of 6-thioguanine-resistant cells per 10(6) cells. Mutant frequency of the cells without NP-III was 8.0, and frequencies of the cells treated with 5, 10 or 20 microM NP-III and UV irradiation were 14.9, 20.6 or 24.7 respectively. Treatment with 20 microM NP-III and UV irradiation decreased the cell number, determined 3 days after the treatment, to 20.8%. These findings indicate that acutely induced oxidative DNA damage including mutagenic 8OHdG is only weakly mutagenic in V79.

Tempel K, Von Zallinger C. **Caffeine-DNA interactions: biochemical investigations comprising DNA-repair enzymes and nucleic acid synthesis.** Z Naturforsch [C] 1997;52(7-8):466-74.
BIOSIS COPYRIGHT: BIOL ABS. Chicken embryo cells were treated with caffeine (0.5-8.0 mm) alone or combined with various chemical and physical DNA- and/or chromatin-interactive agents. Analytical procedures comprised scheduled (SDS) and unscheduled (UDS) DNA synthesis, RNA synthesis (RNS), the activities of O6-alkylguanine-DNA alkyltransferase (AT) and poly (ADP-ribose) polymerase (PARP) as well as nucleoid sedimentation. Additional investigations were done in rat thymic and splenic cells. The effect of caffeine on DNase-I activity served as an in vitro-model system.- When present in the PARP-, SDS-, UDS- and RNS-assays, caffeine inhibited the corresponding tracer (14C-NAD, dT-3H, 3H-U) incorporation in a dose-dependent manner. The AT activity was slightly stimulated. At concentrations of 0.06-0.3 mM, caffeine inhibited DNase-I activity by excess substrate. No specific effects of caffeine could be shown by nucleoid sedimentation.- Besides the reduced permeability of the cells to nucleic acid precursors, the results obtained with the PARP- and DNase-I

assays give evidence for the formation of a DNA-caffeine adduct as a prominent mechanism of cellular caffeine effects including DNA repair inhibition.

Thiruvalluvan M, Manoharan AC, Nagendran N. **Acute and genotoxic effect of cadmium and methyl parathion on Cyprinus carpio var. communis (Linn.).** Geobios 1997;24(4):250-2.
BIOSIS COPYRIGHT: BIOL ABS. Heavy metal, cadmium and organophosphate pesticide methylparathion when tested on Cyprinus carpio var. communis showed that it was more sensitive to latter than to former. Both are genotoxic inducing the formation of micronuclei in the erythrocytes.

Tsutsui T, Hayashi N, Maizumi H, Huff J, Barrett JC. **Benzene-, catechol-, hydroquinoneand phenol-induced cell transformation, gene mutations, chromosome aberrations, aneuploidy, sister chromatid exchanges and unscheduled DNA synthesis in Syrian hamster embryo cells**. Mutat Res 1997;373(1):113-23.
Benzene (71432) and its major metabolic products, hydroquinone (123319) (HQ), phenol (108952) (PH), and catechol (120809) (CA) were examined for their ability to induce cell transformation in the Syrian-hamster-embryo (SHE) cell assay system. SHE cells were treated with benzene, PH, CA, or HQ at 1 to 100 micromolar (mM) for 48 hours. Benzene and PH had little effect on cell growth. CA and HQ inhibited growth at doses greater than 10 or 30mM, respectively. All four chemicals caused morphological transformation and the frequency increased with increasing dose. The highest rate of transformation occurred with CA, followed by HQ, PH, and benzene. All chemicals induced mutations at two loci, and CA was the most potent mutagen. Few chromosomal aberrations occurred when cells were treated for 6 hours with each chemical, but a dose dependent increase in frequencies of chromosome aberrations was induced with PH, CA, and HQ at 24 hours. Benzene had little effect on chromosome aberrations. Slight induction of aneuploidy in the near diploid range occurred in cells treated for 48 hours with benzene, PH, and CA. Benzene had no affect on sister chromosome exchanges, whereas PH, CA, and HQ caused significant increases in sister chromatid exchanges in a dose dependent manner. PH, CA, and HQ induced unscheduled DNA synthesis (UDS), whereas benzene had little affect on UDS. The authors conclude that benzene, HQ, CA, and PH induce cell transformation, gene mutations, and clastogenicity, and CA is the most potent of the four.

Uribe-Luna S, Quintana-Hau JD, Maldonado-Rodriguez R, Espinosa-Lara M, Beattie KL, Farquhar D, Nelson JA. **Mutagenic consequences of the incorporation of 6-thioguanine into DNA.** Biochem Pharmacol 1997;54(3):419-24.
6-Thioguanine (S6G) has been used in the treatment of acute leukemias because of its cytotoxic effect on proliferating leukemic cells. The cytotoxicity of S6G is thought to derive from its incorporation into DNA in place of guanine. The deoxyribonucleoside triphosphate of S6G, SdGTP, is a good substrate for bacterial and human DNA polymerases (Ling et al., Mol Pharmacol 40: 508-514, 1991). Since SdGTP was observed to misincorporate in place of adenine at a greater frequency than did dGTP, it appeared plausible that this analog could produce more subtle effects (mutations) due to mispairing with thymine. To assess whether such mutations occur, SdGTP was incorporated into the lacI gene of phage M13lacISaXb in reactions that omitted dGTP (-G) or dATP (-A). LacI mutation frequency was determined by beta-galactosidase colorimetric staining (inactivation of the lac repressor results in blue plaques in the absence of inducer). When a high concentration of SdGTP (24 microM) was used in the

DNA polymerase reaction, phage infectivity was inhibited. When a relatively low concentration (2.4 nM) was added to the -G and -A reactions, mutagenic effects were observed. DNA sequencing of mutant progeny arising from the -G + S6G reaction revealed C-to-T base transitions and some C-to-A transversions. Similarly, the presence of SdGTP in the -A reactions led to mutants with T-to-C transitions. No insertions or deletions were observed. These data indicate that mispairing of S6G with thymine leads to mutagenic effects in this assay.

Valverde M, Lopez MD, Lopez I, Sanchez I, Fortoul TI, Ostrosky-Wegman P, Rojas E. **DNA damage in leukocytes and buccal and nasal epithelial cells of individuals exposed to air pollution in Mexico city.** Environ Mol Mutagen 1997;30(2):147-52.
BIOSIS COPYRIGHT: BIOL ABS. There is an increased interest in using biological markers to monitor individuals for possible exposure to environmental toxicants. Test systems which permit the sensitive defection of DNA damage and DNA repair are critically important in such studies. The single cell gel electrophoresis (SCG) assay is a rapid and a sensitive method for the evaluation of DNA damage at the single cell level, providing information on the occurrence of DNA single-strand breaks and alkali labile sites using alkaline conditions. In this study, the differences in the basal level of DNA damage between young adults from the south (exposed principally to high levels of ozone) and young adults from the north (exposed principally to hydrocarbons and particles) of Mexico City were investigated by the SCG assay using three different cell types (leukocytes and nasal and buccal epithelial cells). We found an increased DNA migration in blood leukocytes and nasal cells from individuals who live in the southern part of the city compared to those living in the northern port; however, no differences were observed for buccal epithelial cells. These results show the feasability of using the SCG assay to evaluate DNA damage in different tissues and its great potential for use in the monitoring of humans potentially exposed to genotoxic pollutants.

Van Goethem F, Lison D, Kirsch-Volders M. **Comparative evaluation of the in vitro micronucleus test and the alkaline single cell gel electrophoresis assay for the detection of DNA damaging agents: genotoxic effects of cobalt powder, tungsten carbide and cobalt-tungsten carbide.** Mutat Res 1997;392(1-2):31-43.
Although it is well known that micronuclei may arise from either DNA breakage leading to acentric chromosome fragments or from chromosome/chromatid lagging in anaphase, the ratio between the amount of DNA breakage induced and the frequency of micronuclei expressed in the following interphase is unclear. With the development of the alkaline single cell gel electrophoresis assay, which measures single strand and/or double strand breaks in a cell by cell approach, it is new possible to address this question at the cellular level. We therefore compared the genotoxic potential of pure cobalt powder (Co) and a cobalt-containing alloy, cobalt-tungsten carbide (WC-Co), involved in specific lung disorders, in parallel with the alkaline single cell gel electrophoresis (SCGE) assay (comet assay) and the cytokinesis-blocked micronucleus (MN) test, both carried out in vitro on isolated human leukocytes. The comet assay indicated that the WC-Co mixture produced a higher level of DNA damage than Co alone; WC alone was not able to induce a dose-dependent DNA breakage effect as was seen for Co and WC-Co. Results from the MN test confirmed these observations. It was clear that the clastogenic property of Co-containing dust is significantly enhanced when the Co metal is mixed with WC and suggested that their physicochemical characteristics may act as one of the important parameters

responsible for the increased incidence of lung cancers observed in the population of hard metal workers. In agreement with data obtained in the same laboratory on liposoluble chemicals (PCBs and chlorinated aliphatic hydrocarbons) and from the literature, the results indicate that both the comet assay and the micronucleus test were able to detect differences in the genotoxic potential of the compounds studied. Although the micronucleus test seemed to be less sensitive to assess a synergistic DNA damaging potential of the mixture involved, it detects chromosomal aberrations (chromosome/genome mutations) and not just repairable DNA breakage or alkali-labile sites. Combination of the comet assay and the in vitro MN test might therefore be recommended for genotoxins to understand the mechanisms underlying mutagenicity and to assess the lowest efficient dose.

Venkatachalam P, Jayabalan N. **Frequency and spectrum of viable mutations in groundnut induced by physical and chemical mutagens.** Crop Res 1997;14(1):61-75.
BIOSIS COPYRIGHT: BIOL ABS. Viable mutations exhibiting variability in their morphology such as plant types, growth habit, leaf modifications, flower and pod characters mutants were identified and isolated from two genotypes of groundnut from M2 generation, after treatment with different doses/ concentrations of physical as well as chemical mutagens viz., gamma rays, EMS and SA. Modification in plant types like dwarf, tall, busy, disease resistance, erect and number of leaves were most frequent. Both early and late flowering mutants were observed. A number of sterile mutants also were noticed in all mutagenic treatments. Some of the viable mutations of interest realized were those with pod character such as smooth surface, bold, medium size, deep construction pod and without beak noticed in both the cultivars. In general, the frequency of viable mutations was more in VRI-2 than in TMV-7 cultivar. The distinct viable mutants were advanced to M3 generation.

Verri T, Argenton F, Tomanin R, Scarpa M, Storelli C, Costa R, Colombo L, Bortolussi M. **The bacteriophage T7 binary system activates transient transgene expression in zebrafish (Danio rerio) embryos**. Biochem Biophys Res Commun 1997;237(3):492-5.
CBAC COPYRIGHT: CHEM ABS The bacteriophage T7 binary expression system is widely used in vitro for high level selective expression of cloned genes but its application to in vivo models has not yet been investigated. The present work shows that coinjection into fertilized zebrafish eggs of pE1T7R, an expression plasmid bearing the T7 RNA polymerase gene driven by the cytomegalovirus (CMV) promoter, together with reporter vectors contg. the Escherichia coli lacZ gene driven by the T7 promoter, resulted in the efficient expression of the reporter gene in 24-h mosaic transgenic embryos. Conversely, embryos receiving an unrelated CMV-expression plasmid, instead of pE1T7R, lacked significant reporter gene activity, indicating the strict requirement of T7 polymerase to activate the T7 promoter in these embryos. The present study demonstrates the possibility of applying efficiently the bacteriophage T7 binary system in vivo to a vertebrate model.

Vrzoc M, Petras M. **Comparison of DNA damage in peripheral blood and spleen lymphocytes using the single-cell gel assay.** Mutat Res 1997;379(2):263-9.
The alkaline single-cell gel (SCG) or 'comet' assay has been applied to the detection of DNA damage from a number of chemical and biological factors in vivo and in vitro. In the past, a number of cell types has been used with peripheral blood lymphocytes being the most readily accessible. This study was designed to determine whether lymphocytes sequestered in the spleen might prove more sensitive to

DNA damage than those in the peripheral circulation. This would result in a more effective SCG assay. Baseline DNA length to width ratios for peripheral blood and splenic lymphocytes did not differ significantly from each other (1.27 and 1.21, respectively). Neither did ratios of lymphocytes from the two sources, sampled 20 and 48 h after injection with 100 mg/kg methyl methanesulfonate (MMS) (3.81 and 3.62 at 20 h, respectively; and 1.96 and 2.21 at 48 h, respectively). Recovery from MMS damage at 168 h postinjection was also not different in the two groups of cells (1.13 and 1.16, respectively). However, an examination of cell profiles of DNA damage showed that splenic lymphocytes had a significantly higher percentage of damaged cells (63.33%) than did peripheral blood lymphocytes (40.67%) 48 h postinjection. Of the hypotheses proposed for this difference, the most likely seems to involve the different proportions of B- and T-lymphocytes present in the peripheral blood and the spleen. Since the difference between peripheral blood and splenic lymphocytes was seen only at 48 h postinjection, the use of splenic lymphocytes in the SCG assay is not advantageous under most circumstances.

Vrzoc M, Petras ML. **Comparison of alkaline single cell gel (Comet) and peripheral blood micronucleus assays in detecting DNA damage caused by direct and indirect acting mutagens.** Mutat Res 1997;381(1):31-40.
The alkaline single cell gel (SCG) or 'comet' and peripheral blood micronucleus (pbMN) assay have been used to compare the effects of the direct acting mutagens, methyl methanesulfonate (MMS) and N-nitroso-N-methylurea (NMU), and the indirect acting mutagens, benzo[a]pyrene (BAP), cyclophosphamide (CP) 9, 10-dimethyl-1,2-benzanthracene (DMBA), and mitomycin C (MMC) in an inbred house mouse (Mus domesticus) strain. The alkaline SCG assay was able to detect DNA damage from direct acting mutagens. However, it appears that, even at the highest concentrations tested, the SCG assay was not able to detect DNA damage caused by 3 of 4 indirect acting mutagens tested. The exception was BAP. The pbMN assay was sensitive to DNA damage caused by both groups of mutagens. Multiple injections did not increase the sensitivity of the SCG assay to the indirect acting mutagen CP. Further, simultaneous injections of CP and MMS, in one experiment, resulted in significantly lower ($p < 0.05$) average DNA ratios and micronucleated polychromatic erythrocyte counts than those obtained after treatment with MMS alone. Although the SCG assay has been shown to be sufficiently sensitive to detect DNA damage caused by both direct and indirect acting mutagens in deermice (Peromyscus maniculatus) and bullheads (Ameiurus nebulosus), similar results are not seen in the inbred house mouse strain tested.

Wagner ED, Repetny K, Tan JS, Gichner T, Plewa MJ. **Mutagenic synergy between paraoxon and mammalian or plant-activated aromatic amines.** Environ Mol Mutagen 1997;30(3):312-20.
Paraoxon (diethyl-p-nitrophenylphosphate) is the toxic, but non-mutagenic metabolite of the organophosphorus ester (OP) insecticide parathion. Although this agent has been used as a deacetylase inhibitor in many studies, we discovered a mutagenic synergy with paraoxon and plant-activated m-phenylenediamine or with direct-acting 2-acetoxyacetylaminofluorene in Salmonella typhimurium cells [Gichner T et al. (1996): Environ Mol Mutagen 27; 59-66]. In the present study, mammalian-activated m-phenylenediamine, o-phenylenediamine, p-phenylenediamine, benzidine, 2,3-diaminophenazine or 2-aminofluorene, as well as plant-activated benzidine or 2-aminofluorene expressed an elevated mutagenic potency when assayed with S. typhimurium strain YG1024 in the presence of paraoxon. Under non-

toxic conditions, paraoxon amplified the S. typhimurium mutant yield induced by these aromatic amines between 1.9-fold and 8.4-fold. Spectrophotometric analysis demonstrated that the rate of degradation of 2-acetoxyacetylaminofluorene was not significantly different in phosphate buffer with or without paraoxon or with S. typhimurium cytosol with or without paraoxon. Also paraoxon-mediated mutagenic synergy does not appear to be due to a direct reaction with aromatic amines. Mutagenic synergy between aromatic amines and OP oxon products may be a cause of concern because people are chronically exposed to environmental and dietary aromatic amines, and a significant segment of the U.S. population tested positive for OP insecticide residues.

Walker KA, Jennings CD, Pulliam J, Ogburn C, Martin GM, Urano M, Turker MS. **A cell line selected for resistance to ionizing radiation exhibits cross resistance to other genotoxic agents and a mutator phenotype for loss of heterozygosity events.** Somat Cell Mol Genet 1997;23(2):111-21.
An ionizing radiation resistant derivative was obtained from the mouse P19H22 (aprt hemizygote) embryonal carcinoma cell line by repeated exposure to 137Cs gamma radiation. Ionizing radiation resistance in the 6Gy-R cell line was not correlated with a failure to undergo cell cycle arrest or a loss of the p53 response after exposure to 137Cs gamma radiation. Moreover, the cells did not display increased resistance to bleomycin, a double strand break inducing agent. However, the cells did display increased resistance to ultraviolet radiation, ethyl methanesulfonate, and 95% oxygen. A mutational analysis demonstrated a > 700 fold-fold increase in the frequency of aprt mutants for the 6Gy-R cells, but no change in the frequency of hprt or dhfr mutants. A molecular analysis suggested that the aprt mutations in the 6Gy-R cells arose by recombinational events. A possible association between radiation resistance, DNA repair, and a mutator phenotype for large-scale mutational events is discussed.

Weinfeld M, Lee J, Ruiqi G, Karimi-Busheri F, Chen D, Allalunis-Turner J. **Use of a postlabelling assay to examine the removal of radiation-induced DNA lesions by purified enzymes and human cell extracts.** Mutat Res 1997;378(1-2):127-37.
We have used a 32P-postlabelling assay to examine the activity of purified Esherichia coli endonuclease IV, human apurinic/apyrimidinic endonuclease I and human cell-free extracts towards irradiated DNA. The assay can detect thymine glycols, 3'-phosphoglycolate groups and at least one other major lesion that has yet to be fully characterized. It was observed that endonuclease IV removed the phosphoglycolates and the uncharacterized lesion(s) suggesting that the latter are abasic sites with modified deoxyribose residues. The purified human enzyme acted only on the phosphoglycolate residues. Cell-free extract, prepared from A549 lung carcinoma cells by sonication or treatment with toluene, efficiently removed the phosphoglycolate and unknown lesions, but was less reactive towards thymine glycols. The extract was completely inactivated by heating at 60 degrees C for 10 min. Removal of the unknown product and phosphoglycolate did not require magnesium, but 1 mM EDTA did inhibit release of the latter. The cell-free extract exhibited substantially more activity towards native than heat-denatured DNA. A comparison of extracts prepared from 4 cell lines displaying a range of radiosensitivities, including an ataxia telangiectasia cell line, showed that all contained similar levels of repair activity towards the detectable lesions.

Woolard DL, Koscica T, Rhodes DL, Cui HL, Pastore RA, Jensen JO, Jensen JL, Loerop WR, Jacobsen RH, et al. **Millimeter wave-induced vibrational modes in DNA as a possible alternative to animal**

tests to probe for carcinogenic mutations. J Appl Toxicol 1997;17(4):243-6.
CBAC COPYRIGHT: CHEM ABS Developing methods for alternative testing is increasingly important due to dwindling funding resources and increasing costs assocd. with animal testing and legislation. The authors propose to test the feasibility of a new and novel method for detecting DNA mutagenesis using millimeter wave spectroscopy. Although millimeter wave spectroscopy has been known since the 1950s, the cost was prohibitive and studies did not extend to large biol. proteins such as DNA. Recent advances have made this technol. feasible for developing lab. and field equipment. The authors present preliminary findings for lesion-induced vibrational modes in DNA obsd. from 80 to 1000 GHz (GHz). These findings suggest that there are vibrational modes that can be used as identification resonances. These modes are assocd. with localized defects of the DNA polymers. They are unique for each defect/ lesion, and should be easy to detect. The authors described a field-detecting detector based on the local modes.

Woollons A, Clingen PH, Price ML, Arlett CF, Green MH. **Induction of mutagenic DNA damage in human fibroblasts after exposure to artificial tanning lamps.** Br J Dermatol 1997;137(5):687-92.
There is increasing concern about the adverse health effects associated with the use of sunbeds, particularly with respect to skin photocarcinogenesis. The induction of mutagenic DNA damage is a prerequisite for the development of skin tumours, and it is well established that direct types of damage such as cyclobutane pyrimidine dimers (CPDs) give rise to mutations in tumour suppressor genes and oncogenes. In addition, ultraviolet radiation may induce indirect types of DNA damage, including oxidative products, which are also potentially mutagenic. By using specific DNA repair enzymes (T4 endonuclease V and endonuclease III) and the comet assay we have been able to detect the induction of CPDs, oxidized or hydrated pyrimidine bases and single-strand breaks in cultured human fibroblasts (MRC-5) after exposure for between 15 s and 20 min on two different commercial sunbeds containing Philips 'Performance' 100W-R or Philips TL80W/10R lamps. The ratio of endonuclease III to T4 endonuclease V sensitive sites varied substantially between the two lamps and was 3.3% and 18%, respectively. The sunbed containing the 'Performance' 100W-R lamps was as potent at inducing CPDs as was natural sunlight in fine weather. These results establish that commercial tanning lamps produce the types of DNA damage associated with photocarcinogenesis in human cells, and complement epidemiological evidence indicating the potential risk of using sunbeds.

Xie Y, Chen X, Wagner TE. **A ribozyme-mediated, gene knockdown strategy for the identification of gene function in zebrafish.** Proc Natl Acad Sci U S A 1997;94(25):13777-81.
The zebrafish system offers many unique opportunities for the study of molecular biology. To date, only random mutagenesis, and not directed gene knockouts, have been demonstrated in this system. To more fully develop the potential of the zebrafish system, an approach to effectively inhibit the expression of any targeted gene in the developing zebrafish embryo has been developed. This approach uses a transient, cytoplasmic, T7 expression system, injected into the fertilized zebrafish egg to rapidly produce high levels of a ribozyme directed against the mRNA encoded by the targeted gene to inhibit its expression. In a demonstration of this strategy, expression of the recessive dominant zebrafish no tail gene was effectively inhibited by using this strategy to yield a phenotype identical to that resulting from a known defective mutation in this same gene. This, ribozyme-mediated, message deletion strategy may have use in determining the function of genetic coding sequences of unknown function.

Yadollahi-Farsani M, McKenna PG, McKelvey-Martin VJ. **Molecular mechanisms of mutagen hypersensitivity in adenine phosphoribosyl transferase-deficient Friend mouse erythroleukaemia cells.** Br J Biomed Sci 1997;54(3):174-80.
BIOSIS COPYRIGHT: BIOL ABS. Deficiency of the enzyme adenine phosphoribosyltransferase (APRT) has been associated with hypersensitivity to the mutagenic effects of ethyl methanesulphonate (EMS) and 254 nm ultraviolet (UV) radiation in clone 707 of Friend mouse erythroleukaemia (FEL) cells. The molecular nature of spontaneous EMS- and UV-induced mutations in the coding region of hypoxanthine-guanine phosphoribosyltransferase (HPRT) was determined for wild-type FEL cells and two APRT-deficient mutant sub-clones which have significantly reduced ATP pool levels, and are mutagen-hypersensitive. Mis-sense base substitutions were the predominant type of spontaneous mutation. However, exon deletions, possibly involving aberrant splicing of HPRT mRNA, and a non-sense mutation were also observed. EMS-induced mutations in wild-type and APRT-deficient mutant subclones were GC-AT transitions, which is consistent with O6-ethylguanine being the primary pre-mutagenic lesion. All UV-Induced mutations in both cell types were targeted to dipyrimidine sites where the two most common classes of photoproducts (cyclobutane pyrimidine dimers and (6-4) photoproducts) are formed. The similarity in the mutations observed in both cell types indicates that the mutagen hypersensitivity of APRT-deficient cells may be the result of decreased efficiency in the excision repair processes due to reduced levels of ATP.

Yamada M, Matsui K, Sofuni T, Nohmi T. **New tester strains of Salmonella typhimurium lacking O6-methylguanine DNA methyltransferases and highly sensitive to mutagenic alkylating agents.** Mutat Res 1997;381(1):15-24.
Salmonella typhimurium YG7104 and YG7108 are derivatives of the Ames tester strain TA1535, and have chromosomal deletions of the ogtST gene or both the ogtST and adaST genes, respectively. The ogtST and adaST genes encode O6-methylguanine DNA methyltransferases that are involved in the repair of DNA damage caused by alkylating agents. The sensitivities of these strains to 15 mutagens with different structures were tested and compared with those of the parent strain TA1535. Deletion of ogtST or ogtST plus adaST substantially increased the sensitivity of strain TA1535 to the mutagenicity of alkylating agents, such as N-ethyl-N'-nitro-N-nitrosoguanidine, ethyl methanesulfonate or dimethylnitrosamine (DMN). Preincubation of the chemical with S9 mix and bacteria for 20 min at 37 degrees C before pouring them together on agar plates was not necessary to detect the mutagenicity of DMN when strain YG7104 or YG7108 was used as a tester strain. Introduction of plasmid pKM101 did not enhance but rather decreased the sensitivity of YG7104 and YG7108 to alkylating agents. Since the new strains are highly sensitive only to alkylating agents, they will be useful to detect the mutagenicity with high efficiency and to study the mechanism of mutagenesis induced by environmental alkylating agents.

Zha H, Reed JC. **Heterodimerization-independent functions of cell death regulatory proteins Bax and Bcl-2 in yeast and mammalian cells.** J Biol Chem 1997;272(50):31482-8.
The pro-apoptotic protein Bax can homodimerize with itself and heterodimerize with the anti-apoptotic protein Bcl-2, but the significance of these protein-protein interactions remains unclear. Alanine substitution mutations were created in a well conserved IGDE motif found within the BH3 domain of

Bax (residues 66-69) and the resulting mutant Bax proteins were tested for ability to homodimerize with themselves and to heterodimerize with Bcl-2. Correlations were made with cell death induction by these mutants of Bax both in mammalian cells where Bax may function through several mechanisms, and in yeast where Bax may exert its lethal actions through a more limited repertoire of mechanisms perhaps related to its ability to form ion channels in intracellular membranes. Two of the mutants, Bax(D68A) and Bax(E69A), retained the ability to homodimerize but failed to interact with Bcl-2 as determined by yeast two-hybrid assays and co-immunoprecipitation analysis using transfected mammalian cells. The Bax(E69A) protein exhibited a lethal phenotype in yeast, which could be specifically suppressed by co-expression of Bcl-2, despite its failure to dimerize with Bcl-2. Both the Bax(D68A) and Bax(E69A) proteins induced apoptosis when overexpressed in human 293 cells, despite an inability to bind to Bcl-2. Moreover, co-expression of Bcl-2 with Bax(D68A) and Bax(E69A) rescued mammalian cells from apoptosis. In contrast, a mutant of Bax lacking the IGDE motif, Bax(DeltaIGDE), was incapable of either homodimerizing with itself or heterodimerizing with Bcl-2 and was inactive at promoting cell death in either yeast or mammalian cells. Although failing to interact with Bcl-2, the Bax(D68A) and Bax(E69A) mutants retained the ability to bind to Bid, a putative Bax-activating member of the Bcl-2 family, and collaborated with Bid in inducing apoptosis. When taken together with previous observations, these findings indicate that (i) Bax can induce apoptosis in mammalian cells irrespective of heterodimerization with Bcl-2 and (ii) Bcl-2 can rescue both mammalian cells and yeast from the lethal effects of Bax without heterodimerizing with it. However, these results do not exclude the possibility that BH3-dependent homodimerization of Bax or interactions with Bax activators such as Bid may either assist or be required for the cell death-inducing mechanism of this protein.

Zhan DJ, Chiu LH, Von Tungeln LS, Herreno-Saenz D, Cheng E, Evans FE, Heflich RH, Fu PP. **Characterization of DNA adducts in Chinese hamster ovary cells treated with mutagenic doses of 1- and 3-nitrosobenzo[a]pyrene and the trans-7,8-diol-anti-9,10-epoxides of 1- and 3-nitrobenzo[a] pyrene.** Mutat Res 1997;379(1):43-52.

The environmental contaminants 1- and 3-nitrobenzo[a]pyrene (1- and 3-nitro-BaP) are mutagens in Chinese hamster ovary (CHO) cells with exogenous metabolic activation. Previous studies demonstrated the potent direct-acting mutagenicity of the oxidized metabolites, trans-7,8-dihydroxy-anti-9,10-epoxy-7,8,9,10-tetrahydro-1-nitrobe- nzo[a] pyrene (1-NBaPDE) and trans-7,8-dihydroxy-anti-9,10-epoxy-7,8,9, 10-tetrahydro-3-nitrobenzo[a]pyrene (3-NBaPDE), and the partially nitroreduced metabolites, 1- and 3-nitrosobenzo[a]pyrene (1- and 3-NO-BaP). In this study, we have identified the major adduct formed by incubation of calf thymus DNA with 1-NBaPDE and used this standard in conjunction with other adduct standards to characterize the 32P-postlabeled DNA adducts produced by 1- and 3-nitro-BaP metabolites in CHO cultures. The major adduct from 1-NBaPDE exposure was 10-(deoxyguanosin-N2-yl)-7,8,9-trihydroxy-7,8,9,10-tetrahydro-1- nitrobenzo[a]pyrene; from 3-NBaPDE, 10-(deoxyguanosin-N2-yl)-7,8,9-trihydroxy-7,8,9,10-tetrahydro-3- nitrobenzo[a]pyrene; from 1-NO-BaP, 6-(deoxyguanosin-N2-yl)-1-aminobenzo[a]pyrene; and from 3-NO-BaP, 6-(deoxyguanosin-N2-yl)-3-aminobenzo[a] pyrene. For comparison, the adducts formed by trans-7,8-dihydroxy-anti-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]py- rene and the related nitroreduced derivative 6-nitrosobenzo[a]pyrene were also examined. The nitrobenzo[a]pyrene DNA adducts described in this study are proposed to be involved in the mutagenicity of 1- and 3-nitro-BaP upon either oxidative or reductive metabolism.

Zoladek T, Nguyen BN, Jagiello I, Graczyk A, Rytka J. **Diamino acid derivatives of porphyrins penetrate into yeast cells, induce photodamage, but have no mutagenic effect.** Photochem Photobiol 1997;66(2):253-9.
The yeast Saccharomyces cerevisiae was used as a model eukaryotic organism to study the uptake of diamino acid derivatives of porphyrins and their phototoxicity with particular emphasis on possible mutagenic effects. The water-soluble hematoporphyrin derivatives diarginate (HpD[Arg]2) and 1-arginin di(N-amino acid)-protoporphyrinate used in this study are effective photosensitizers in tumor photodynamic therapy. Depending on the amino acid substituent, the porphyrin derivatives differ in their affinity for yeast cells. It is shown that HpD(Arg)2 and PP(Met)2 (Arg)2 penetrate into the yeast cell and are metabolized. Both compounds sensitize yeast cells to photodamage but have no mutagenic effect on nuclear or mitochondrial genomes.

HEPATIC AND RENAL TOXICITY

Conolly RB, Andersen ME. **Hepatic foci in rats after diethylnitrosamine initiation and 2,3,7,8-tetrachlorodibenzo-p-dioxin promotion: evaluation of a quantitative two-cell model and of CYP 1A1/1A2 as a dosimeter.** Toxicol Appl Pharmacol 1997;146(2):281-93.
BIOSIS COPYRIGHT: BIOL ABS. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a potent hepatic tumor promoter in female rats. We used a quantitative, stochastic initiation-promotion model based on R. B. Conolly and J. S. Kimbell (Toxicol. Appl. Pharmacol. 124, 284-295, 1994) to analyze initiation-promotion results from a previously published study (H. C. Pitot et al., Carcinogenesis 8, 1491-1499, 1987) within the context of a negative selection model of tumor promotion. In this model, two types of initiated cells (called A and B cells) are produced by DEN initiation. Visually excellent correspondence between model predictions and data (i.e., foci/cm3 liver and percentage of liver occupied by foci) are obtained when TCDD is described as having dose-responsive effects on division and death (apoptotic) rates of these two cell types. For A cells, both the division and the death rates increase while the difference between division and apoptotic rates decreases. For B cells, the difference between division and apoptotic rates increases, primarily due to a decrease in the apoptotic rate. We also linked these alterations in cell kinetics to a pharmacokinetic model for TCDD incorporating a five subcompartment model of the liver acinus with induction of CYP1A1 and 1A2 genes in the subcompartments. Alterations in A cell kinetics correlate with effects of TCDD in the region most sensitive to induction (subcompartment 5-centrilobular region); B cell dynamics correlate with induction in subcompartments 3-5 (centrilobular and mid-zonal regions). In summary, these modeling exercises show that (1) the two-cell model, without presuming effects of TCDD on the mutation rate of normal hepatocytes, reproduces the data of Pitot et al. (1987) and (2) induction of CYP1A1/1A2 in different regions of the hepatic acinus can be used as a general correlate of these presumed changes in cell growth kinetics.

Indulski JA, Lutz W. **[Biomarkers of hepatotoxic effects: their usefulness in occupational medicine]**. Med Pr 1997;48(2):177-87. (Pol)
BIOSIS COPYRIGHT: BIOL ABS. 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.

Kedderis GL. **Extrapolation of in vitro enzyme induction data to humans in vivo.** Chem Biol Interact 1997;107(1-2):109-21. Enzyme induction generally increases the rate and extent of xenobiotic metabolism in vitro, but physiological constraints can dampen these effects in vivo. Biotransformation kinetics determined in hepatocytes in vitro can be extrapolated to whole animals based on the hepatocellularity of the liver, since the initial velocity of an enzyme-catalyzed reaction is directly proportional to the total enzyme present in the cell. The biotransformation kinetics of various xenobiotics determined with isolated hepatocytes in vitro have been shown to accurately predict pharmacokinetics in whole animals. Analysis of the kinetic data, using physiologically based pharmacokinetics, allows extrapolation of xenobiotic biotransformation across dose routes and species in a biologically realistic context. Several fold variations were observed in the bioactivation of the hepatotoxicant furan by isolated human hepatocytes, due to induction of cytochrome P450 2E1. Extrapolation of these data to humans in vivo showed that furan bioactivation was limited by hepatic blood flow delivery of the substrate. One important consequence of hepatic blood flow limitation is that the amount of metabolite formed in the liver is unaffected by increases in Vmax due to enzyme induction. Therefore, interindividual variations in cytochrome P450 2E1 among human populations would not affect the bioactivation of many rapidly metabolized hazardous chemical air pollutants. The hepatic blood flow limitation of biotransformation is also observed after oral bolus dosing of rapidly metabolized compounds. More slowly metabolized xenobiotics, such as therapeutic agents, are only partially limited by hepatic blood flow and other processes.

Tsutsui H, Matsui K, Kawada N, Hyodo Y, Hayashi N, Okamura H, Higashino K, Nakanishi K. **IL-18 accounts for both TNF-alpha- and Fas ligand-mediated hepatotoxic pathways in endotoxin-induced liver injury in mice.** J Immunol 1997;159(8):3961-7.
When LPS is administered to heat-killed Propionibacterium acnes-primed BALB/c nude mice, they develop endotoxin-induced liver injury. As previously reported, this liver injury can be prevented by treatment with an Ab against IL-18, a novel cytokine with the ability to induce IFN-gamma production and up-regulate functional Fas ligand (FasL) expression. To identify the pathologic role of IL-18 in this liver injury, we investigated the hepatic cytokine network and FasL induction after LPS challenge. After LPS challenge to BALB/c nude mice, their livers expressed IL-12 mRNA, followed by the induction of IFN-gamma and FasL mRNA and then by the late elevation of TNF-alpha mRNA, but stably expressed IL-18 mRNA. The TNF-alpha induction curve had two peaks. The first peak was the result of the direct

reaction to LPS, and the late peak might have been induced, since P. acnes-elicited Kupffer cells showed one-peak TNF-alpha kinetics in response to LPS stimulation in vitro. LPS-activated P. acnes-elicited Kupffer cells secreted both IL-12 and IL-18, as determined by ELISA and bioassay, respectively. The in vivo administration of anti-IL-18 just before an LPS challenge suppressed not only the induction of IFN-gamma and the late TNF-alpha elevation, but also the FasL induction, resulting in the total prevention of liver injury, whereas such an anti-IL-12 treatment did not. Anti-IFN-gamma treatment reduced the late increase in TNF-alpha, but not FasL, resulting in a partial prevention of the liver injury. The administration of anti-TNF-alpha just before elevation of the late TNF-alpha peak also markedly, but incompletely, suppressed the LPS-induced liver injury. These data suggested that IL-18 activates both TNF-alpha- and FasL-mediated hepatocytotoxic pathways in endotoxin-induced liver injury.

IMMUNOTOXICITY

Amersdorfer P, Wong C, Chen S, Smith T, Deshpande S, Sheridan R, Finnern R, Marks JD. **Molecular characterization of murine humoral immune response to botulinum neurotoxin type A binding domain as assessed by using phage antibody libraries.** Infect Immun 1997;65(9):3743-52.
To produce antibodies capable of neutralizing botulinum neurotoxin type A (BoNT/A), the murine humoral immune response to BoNT/A binding domain (H(C)) was characterized at the molecular level by using phage antibody libraries. Mice were immunized with BoNT/A H(C), the spleens were harvested, and single-chain Fv (scFv) phage antibody libraries were constructed from the immunoglobulin heavy and light chain variable region genes. Phage expressing BoNT/A binding scFv were isolated by selection on immobilized BoNT/A and BoNT/A H(C). Twenty-eight unique BoNT/A H (C) binding scFv were identified by enzyme-linked immunosorbent assay and DNA sequencing. Epitope mapping using surface plasmon resonance in a BIAcore revealed that the 28 scFv bound to only 4 nonoverlapping epitopes with equilibrium constants (Kd) ranging from 7.3 x 10(-8) to 1.1 x 10(-9) M. In a mouse hemidiaphragm assay, scFv binding epitopes 1 and 2 significantly prolonged the time to neuroparalysis, 1.5- and 2.7-fold, respectively, compared to toxin control. scFv binding to epitopes 3 and 4 showed no protection against neuroparalysis. A combination of scFv binding epitopes 1 and 2 had an additive effect on time to neuroparalysis, which increased to 3.9-fold compared to the control. The results suggest that there are two productive receptor binding sites on H(C) which lead to toxin internalization and toxicity. Blockade of these two epitopes with monoclonal antibodies may provide effective immunoprophylaxis or therapy against BoNT/A intoxication.

Bezard M, Karlberg AT, Montelius J, Lepoittevin JP. **Skin sensitization to linalyl hydroperoxide: support for radical intermediates.** Chem Res Toxicol 1997;10(9):987-93.
In order to better understand the skin sensitization mechanism of allylic hydroperoxides, linalyl hydroperoxide (1) and several of its potential rearrangement products-epoxylinalool (2), epoxynerol (3), epoxygeraniol (4), and furan (5) and pyran (6) derivatives-were synthesized. The sensitizing properties of these molecules have been screened on mice using the local lymph node assay (LLNA) and further evaluated on guinea pigs using the Freund's complete adjuvant test (FCAT). Linalyl hydroperoxide (1) and linalyl epoxide (2) were found to be sensitizers, while the other compounds were classified as mild sensitizers or nonsensitizers. In the guinea pigs, no cross-reactions were observed between skin sensitizers 1 and 2. Radical-trapping experiments were carried out on linalyl hydroperoxide (1) using

TTBP as trapping agent and Fe(3+)-TPP as radical inducer. The major reaction taking place is the formation of a furan ring by intramolecular reaction of the oxygen-centered radical with the isoprenyl double bond with the formation of a tertiary radical. Reaction of this intermediate with radicals derived from TTBP gave compounds 10a,b in 25% yield. The second important reaction, accounting for 14%, is taking place on the allylic double bond with the formation of a less stable primary radical which is not trapped by a TTBP-derived radical but by a hydroxy radical to give a mixture of epoxides 3 and 4. These results are in favor of the formation of a carbon-centered reactive radical as intermediate in the skin sensitization to linalyl hydroperoxide.

Burchiel SW, Kerkvliet NL, Gerberick GF, Lawrence DA, Ladics GS. **Assessment of immunotoxicity by multiparameter flow cytometry.** Fundam Appl Toxicol 1997;38(1):38-54.
Flow cytometry is a unique technology useful in the examination of effects of immunotoxic agents on target cells of the immune system. The purpose of this workshop was to provide an overview of the use of flow cytometry in new and established models of immunotoxicity, with emphasis on the potential applications, assay validation, and potential pitfalls. This overview begins with a discussion of methods useful in the assessment of Ca2+-dependent mechanisms of lymphoid cell activation in surface marker-defined human B cells, T cells, and monocytes. A discussion of the use of flow cytometry in analysis of apoptosis is also presented in this paper. The second paper presents data on the development and use of flow cytometry as an alternative to a Cr51 release assay for an assessment of cytotoxic T cell activation. The use of surface markers for characterizing and distinguishing the effects of chemical irritants from sensitizers is next presented, followed by an overview of the use of fluorescent probes to assess cell thiol status and overall oxidant-induced injury to lymphoid cells. Finally, an interlaboratory study designed to compare and evaluate the use of flow cytometry procedures in rat splenic cell subtyping is presented. Overall, these studies demonstrate the utility of flow cytometry assays in immunotoxicologic research, but further efforts are needed in the validation of many of these assays for routine use in immunotoxicologic testing.

Dunn RD, Weston KM, Longhurst TJ, Lilley GG, Rivett DE, Hudson PJ, Raison RL. **Antigen binding and cytotoxic properties of a recombinant immunotoxin incorporating the lytic peptide, melittin.** Immunotechnology 1996;2(3):229-40.
BACKGROUND: The majority of immunotoxins studied to date incorporate toxins that act in the cytosol and thus need to be endocytosed by the target cell. An alternative strategy for immunotoxin development is the use of membrane active toxins, such as the pore-forming proteins. Melittin, a 26 amino acid cytolytic peptide from bee venom, is such a protein. OBJECTIVES: We report here the construction, production and functional analysis of a recombinant immunotoxin obtained by fusion of genes which encode an antibody fragment (scFv) with an oligonucleotide encoding melittin. STUDY DESIGN: The antibody fragment was derived from a murine monoclonal antibody, K121, which recognises a specific epitope (KMA) expressed on the surface of human kappa myeloma and lymphoma cells, and on human free kappa Bence Jones protein (BJP). Melittin is a 26-amino acid, membrane-lytic peptide which is a major component of bee venom. The scFv of K121 was constructed by PCR to link VH and VL genes via an oligonucleotide which encodes a flexible, hydrophilic peptide. An oligonucleotide encoding melittin and the peptide marker sequence FLAG was fused to the scFv construct using a similar linker peptide. The gene construct (scFv-mel) was inserted into the secretion

vector pPOW and expressed in Escherichia coli (TOPP2). RESULTS: Expression of the recombinant scFv-mel gene and purification of the protein product was monitored by Western blot analysis. Following purification by anti-FLAG affinity chromatography, the recombinant immunotoxin (scFv-mel) was assessed for antigen binding and for cytotoxic activity by flow cytometry using antigen-expressing and non-expressing cell targets. The scFv-mel was found to exhibit binding and killing properties consistent with the specificity of the original K121 antibody. Moreover, the cytolytic activity of the scFv-mel was significantly greater on a molar basis than that of native melittin alone. CONCLUSION: The data presented here constitute the first report of a melittin-based recombinant immunotoxin and demonstrate that such a membrane active immunotoxin can be synthesised in a bacterial expression. Linking of melittin to an antibody fragment overcame the non-specific toxicity of melittin as the recombinant immunotoxin exhibited specific toxicity towards antigen-bearing target cells. The observation that the immunotoxin exhibited enhanced cytotoxic activity over the free toxin indicates the potential of this approach for the development of an effective therapeutic agent.

Eperon S, De Groote D, Werner-Felmayer G, Jungi TW. **Human monocytoid cell lines as indicators of endotoxin: comparison with rabbit pyrogen and Limulus amoebocyte lysate assay.** J Immunol Methods 1997;207(2):135-45.
The aim of this study was to develop an in vitro test system for pyrogenic substances. Three clones derived from human monocytoid cell lines, which were selected by their high sensitivity to lipopolysaccharide (LPS), were assessed for tumor necrosis factor (TNF) production. Their response to pyrogen-containing samples was compared with that in a Limulus amoebocyte lysate assay and the rabbit pyrogen test. We show here that the induction of TNF in these clones is a valid in vitro alternative to determine endotoxin in commercial preparations requiring pyrogenicity testing. Cell clones derived from Mono Mac 6 (MM6 2H8 and MM6 4B5) responded to sub-ng/ml concentrations of complete rough-strain and smooth-strain LPS, to ng/ml concentrations of diphosphoryl-lipid A, and to microgram/ml concentrations of monophosphoryl-lipid A and to detoxified LPS. Cells reacted to > or = 1 microgram/ml lipoteichoic acid by TNF production, and were relatively insensitive to toxic shock syndrome toxin-1 (TSST-1) and to muramyl dipeptide adjuvant peptide. The reaction pattern of a clone derived from THP-1 (THP-1 1G3) was in general, similar to that of the MM6 clones, except that THP-1 1G3 failed to react to diphosphoryl-lipid A. When tested on commercial samples destined for parenteral use, there was a close correlation between a sensitive Limulus amoebocyte lysate (LAL) test and the cell culture test on the one hand, and between the pyrogen test and the cell culture test on the other hand. The data suggest that this cell-based test is able to recognize pyrogens derived from gram-negative organisms in test samples with appropriate sensitivity and specificity. This test appears to be able to eliminate some of the false-positive data obtained in the LAL test.

Faguet GB, Agee JF. **The chronic lymphocytic leukemia antigen (cCLLa) as immunotherapy target: assessment of LD50 and MTD of four ricin-based anti-cCLLa immunotoxins (ITs) in Balb/c mice.** Leuk Lymphoma 1997;25(5-6):531-7.
The chronic lymphocytic leukemia (CLL) antigen (cCLLa) is a promising immunotherapy target given its disease-restricted expression, its highest prevalence among CLL surface antigens, and its lack of expression by normal T- and B-lymphocytes. The objectives of this study were to assess the 50% lethal dose (LD50) and the maximum tolerated dose (MTD) in Balb/c mice of four anti-cCLLa immunotoxins

(ITs) derived from the intact monoclonal antibody (MoAb) or its Fab fraction, each conjugated to either ricin chain-A (RTA) or its deglycosylated derivative (dgA). The IgG fraction of anti-cCLLa monoclonal antibody CLL2m and its Fab fraction were conjugated to RTA or dgA to generate four ITs: IgG/RTA, IgG/dgA, Fab/RTA and Fab/dgA. Progressive concentrations of each IT (ranging between 2.60 mg/kg and 100.00 mg/kg) were injected intravenously into groups of 5 mice each. After injection, mice were monitored daily for 10 days for survival. Observed mortality data in each group were matched to those in Weil's tables for estimating LD50 (mg/kg) from the moving average interpolation method. Estimated LD50 (in mg/kg) were: IgG/RTA, 13.33; Fab/RTA, 25.53; IgG/dgA, 55.33; Fab/dgA, 55.33. Their respective MTD (mg/kg), defined as the highest dose level survived by all mice, were 8.78, 13.17, 29.63 and 29.63. Depending on the animal-to-human extrapolation method used, the calculated LD50 and MTD in humans ranged from 1.2 mg/kg and 0.8 mg/kg (IgG/RTA), to 55.6 mg/kg and 36.9 mg/kg (IgG/dgA and Fab/dgA), respectively. The following conclusions are drawn. 1. Antibody valence exerted little influence on either the LD50 or the MTD; 2. The LD50 to MTD ratios were approximately 2:1; 3. dgA-derived ITs were approximately one half as toxic as their RTA-derived counterparts; and 4. Extrapolation of LD50 and MTD mouse data to humans resulted in dose levels comparable to or exceeding those reported in most IT human trials. These data suggest the suitability of anti-cCLLa ITs for clinical immunotherapy trials.

Francisco JA, Gawlak SL, Miller M, Bathe J, Russell D, Chace D, Mixan B, Zhao L, Fell HP, Siegall CB. **Expression and characterization of bryodin 1 and a bryodin 1-based single-chain immunotoxin from tobacco cell culture.** Bioconjug Chem 1997;8(5):708-13.
BIOSIS COPYRIGHT: BIOL ABS. Bryodin 1 (BD1) is a potent ribosome-inactivating protein (RIP) isolated from the plant Bryonia dioica. It is relatively nontoxic in rodents (LD50 > 40 mg/kg) and represents a potential improvement over other RIPs and bacterial toxins that have been used in immunotoxins. Recombinant BD1, expressed in Escherichia coli, localizes to insoluble inclusion bodies necessitating denaturation and refolding steps to generate active protein. In this report, BD1 was expressed as a soluble recombinant protein in tobacco cell culture (ntBD1) and purified to near homogeneity with yields of up to 30 mg/(L of culture). The protein synthesis inhibition activity of ntBD1 was identical to that of both native BD1 isolated from the roots of B. dioica and recombinant BD1 expressed in E. coli. Toxicology analysis showed that ntBD1 was well tolerated in rats at doses that cannot be achieved with most other toxin components of immunotoxins. Additionally, a single-chain immunotoxin composed of BD1 fused to the single-chain Fv region of the anti-CD40 antibody G28-5 (ntBD1-G28-5 sFv) was expressed in tobacco tissue culture as a soluble protein and was specifically cytotoxic toward CD40 expressing non-Hodgkin's lymphoma cells in vitro. These data indicate that tobacco tissue culture is a viable system for soluble expression of BD1 and BD1-containing immunotoxins.

Johnson R, Macina OT, Graham C, Rosenkranz HS, Cass GR, Karol MH. **Prioritizing testing of organic compounds detected as gas phase air pollutants: structure-activity study for human contact allergens.** Environ Health Perspect 1997;105(9):986-92. Organic compounds that are used or generated anthropogenically in large quantities in cities can be identified through their presence in the urban atmosphere and in air pollutant source emissions. Compounds identified by this method were screened to evaluate their potential to act as contact allergens. The CASE and MULTICASE computer

programs, which are based on the detection of structure-activity relationships (SAR), were used to evaluate this potential. These relationships first are determined by comparing chemical structures to biological activity within a learning set comprised of 458 compounds, each of which had been tested experimentally in human trials for its sensitization potential. Using the information contained in this learning set, CASE and MULTICASE predicted the activity of 238 compounds found in the atmosphere for their ability to act as contact allergens. The analysis finds that 21 of 238 compounds are predicted to be active contact allergens (probability >0.5), with potencies ranging from mild to very strong. The compounds come from chemical classes that include chlorinated aromatics and chlorinated hydrocarbons, N-containing compounds, phenols, alkenes, and an S-containing compound. Using the measured airborne concentrations or emission rates of these compounds as an indication of the extent of their use, together with their predicted potencies, provides an efficient method to prioritize the experimental assessment of contact sensitization of untested organic compounds that can be detected as air pollutants.

NEUROTOXICITY

Sims TN, Goes NB, Ramassar V, Urmson J, Halloran PF. **In vivo class II transactivator expression in mice is induced by a non-interferon-gamma mechanism in response to local injury.** Transplantation 1997;64(12):1657-64.
BACKGROUND: Tissue injury induces MHC class II expression, which could be important in the recognition of that tissue as an allograft. The class II transcriptional activator (CIITA) is the major regulator of basal and induced MHC class II expression and is essential for antigen presentation. The role of CIITA in the induction of class II by tissue injury is unknown. In this study, we examined CIITA induction in the course of acute ischemic or toxic renal injury in mice, including the role of interferon (IFN)-gamma and of the transcription factor, interferon regulatory factor (IRF)-1. METHODS: Kidneys were injured by ischemia or by gentamicin toxicity and were then studied for changes in gene expression using Northern blot, reverse transcriptase-polymerase chain reaction, radioimmunoassay, and tissue staining. We compared wild-type (WT) mice to IFN-gamma knockout (GKO) or IRF-1 knockout mice. RESULTS: Ischemic injury induced CIITA and class II expression in the kidney, in WT and GKO mice. Gentamicin injury also induced both CIITA and class II expression, independent of IFN-gamma, in WT and GKO mice. After ischemic injury, the induction of class II protein levels and CIITA and class II mRNA levels were induced, to a lesser degree, in IRF-1 knockout mice. CONCLUSIONS: These data indicate that CIITA is induced by tissue injury, and probably accounts for class II induction during tissue injury. CIITA induction by injury is largely IFN-gamma independent but requires IRF-1. The similarities of the pattern of CIITA and class II induction in ischemic and toxic injury suggest that this is a stereotyped response of injured tissue and not a consequence of a particular mechanism of injury.

Ackermann EJ, Taylor P. **Nonidentity of the alpha-neurotoxin binding sites on the nicotinic acetylcholine receptor revealed by modification in alpha-neurotoxin and receptor structures.** Biochemistry 1997;36(42):12836-44.
BIOSIS COPYRIGHT: BIOL ABS. alpha-Neurotoxins constitute a large family of polypeptides that bind with high affinity to the nicotinic acetylcholine receptor (nAChR). Using a recombinant DNA-derived alpha-neurotoxin (Naja mossambica mossambica, NmmI) and mouse muscle nAChR expressed

transiently on the surface of HEK 293 cells, we have delineated residues involved in the binding interaction on both the alpha-neurotoxin and the receptor interface. Several of the studied NmmI mutations, including two residues conserved through the alpha-neurotoxin family (K27 and R33), resulted in substantial decreases in the binding affinity. We have also examined 23 mutations located on the receptor alpha subunit and have identified 4 positions that appear to be important to NmmI recognition. These determinants represent a conserved aromatic residue (Y190), two positions where neuronal and muscle receptors differ (V188 and P197), and a negatively charged residue (D200). Unlike many of the nAChR agonists and antagonists which bind to alphadelta and alphagamma binding sites on the receptor with different affinities, the wild-type NmmI-wild-type nAChR interaction showed a single affinity. However, by mutating critical toxin or receptor residues, we were able to produce site-selectivity between the alphagamma and alphadelta interfaces. These results suggest a nonequivalence in the binding interaction at the two sites, sensitive to discrete structural changes at key contact points on either the toxin or the receptor protein, and importance of delta and gamma receptor subunits in governing binding affinity.

Acuna MC, Diaz V, Tapia R, Cumsille MA. **[Assessment of neurotoxic effects of methyl bromide in exposed workers]**. Rev Med Chil 1997;125(1):36-42. (Spa)
BACKGROUND: Methyl bromide is an aliphatic hydrocarbon derivative used as a pesticide that causes skin, kidney, respiratory, liver and neurological damage. AIM: To assess the neurological and psychiatric damage caused by methyl bromide in exposed workers of seed and fruit export industries in a rural area near Santiago. SUBJECTS AND METHODS: We studied prospectively 15 male middle age workers before and after a fumigation period with methyl bromide, that lasted two to four weeks. According to the initial assessment, 5 of these subjects had a chronic exposure to the chemical. As controls, 10 non exposed workers matched for age, sex and working conditions were studied in two occasions. The evaluation included the WHO Neuro Behavior Core Test Battery, dynamometric and vibrator assessment of peripheral nerve function, the Nothingham test for psychological functioning and Titmus test for visual acuity. Methyl bromide levels were measured in blood and urine. RESULTS: Blood methyl bromide levels increased from 13.3 to 30 mg/dl after exposure. Symptoms that appeared with a higher frequency in exposed workers were insomnia, headache, paresthesiae, mood changes and loss of memory and concentration. In these subjects, the threshold for the Vibraton test increased from 2.4 to 2.85 sec, dynamometry showed a strength reduction in the right side from 51.4 to 47.2 kg and there was an increase in the score for negative auto-perception in the Nothingham test from 11.2 to 13.6. No deterioration in these tests were observed in unexposed workers. CONCLUSIONS: Acute and chronic methyl bromide exposure causes important psychological and neurological derangement.

Baird SJ, Catalano PJ, Ryan LM, Evans JS. **Evaluation of effect profiles: functional observational battery outcomes.** Fundam Appl Toxicol 1997;40(1):37-51.
The Functional Observational Battery (FOB) is a neurotoxicity screening assay composed of 25-30 descriptive, scalar, binary, and continuous endpoints. These outcomes have been grouped into six biologically logical domains as a means to interpret the neuroactive properties of tested chemicals (V. C. Moser, 1992, J. Am. Coll. Toxicol. 10(6), 661-669). However, no data-based exploration of these functional domains has been done. We investigated the degree to which experimental data correspond to the domain groupings by examining severity scores from 10 chemicals tested using a standardized

protocol for acute exposure (V. C. Moser et al., 1995, J. Toxicol. Environ. Health 45, 173-210) and identifying endpoint groupings (factors) that best describe the interrelationships in the data, allowing a statistical assessment of whether the FOB endpoints break into domains. We also used a standard measure of bivariate association to confirm the results of the factor analysis. Our results show that while there are clear relationships among variables that compose some domains, there is often substantial correlation among endpoints in different domains. In addition, we investigated a related issue concerning the relative power of the chosen endpoint groupings for identifying significant domain effects. Results from a randomization analysis of the 10 chemicals suggest that the neurophysiologic domain structuring may provide some degree of statistical efficiency for identifying effects.

Barth AL, Dugas JC, Ngai J. **Noncoordinate expression of odorant receptor genes tightly linked in the zebrafish genome**. Neuron 1997;19(2):359-69.
CBAC COPYRIGHT: CHEM ABS We have characterized the organization and expression of odorant receptor genes clustered within .apprx.100 kb of the zebrafish genome. Phys. anal. of this genomic region reveals that the receptor genes are tightly linked in tandem arrays. The expression patterns of these genes were evaluated during development as well as in the adult olfactory epithelium. Highly related genes from this array are expressed individually in different olfactory neurons, suggesting that the discriminatory capacity of the vertebrate olfactory system has been maximized by segregating the most similar receptors into distinct cellular pathways. Furthermore, genes from this cluster are activated at different times of development. Together, these results indicate that genomically linked odorant receptor genes are not coordinately regulated.

Brown DR, Herms JW, Schmidt B, Kretzschmar HA. **PrP and beta-amyloid fragments activate different neurotoxic mechanisms in cultured mouse cells.** Eur J Neurosci 1997;9(6):1162-9.
Alzheimer's disease and prion diseases such as Creutzfeldt-Jakob disease are caused by as yet undefined metabolic disturbances of normal cellular proteins, the amyloid precursor protein and the prion protein (PrP). Synthetic fragments of both proteins, beta-amyloid 25-35 (betaA25-35) and PrP106-126, have been shown to be toxic to neurons in culture. Cell death in both cases occurs by apoptosis. Here we show that there are considerable differences in the mechanisms involved. Thus, PrP106-126 is not toxic to cortical cell cultures of PrP knockout mouse neurons whereas betaA25-35 is. The toxicity of both peptides involves Ca2+ uptake through voltage-sensitive Ca2+ channels but only PrP106-126 toxicity involves the activity of NMDA receptors. The toxicity of betaA25-35, but not PrP106-126, is attenuated by the action of forskolin. These results indicate that PrP106-126 and PA25-35 induce neuronal apoptosis through different mechanisms.

Bruinink A, Sidler C. **The neurotoxic effects of ochratoxin-A are reduced by protein binding but are not affected by l-phenylalanine.** Toxicol Appl Pharmacol 1997;146(2):173-9.
BIOSIS COPYRIGHT: BIOL ABS. Recent in vivo investigations indicate that the mycotoxin ochratoxin A (OTA) is a neurotoxicant during prenatal stages. In line with in vivo data, in our embryonic chick brain and neural retina cell cultures the markers for neuritic outgrowth and differentiation (NF68 and 160 kDa, MAP2 and MAP5) were especially negatively affected. In vivo OTA is nearly completely bound to serum constituents. In our culture system binding of OTA to BSA evoked a significant shift of the concentration-effect relationships in meningeal and brain cell cultures. As a result of the albumin

binding the OTA IC5 and IC50 values of all parameters increased by nearly the same value (about 15-fold in brain and 32-fold in meningeal cell cultures). One of the mechanisms responsible for OTA toxicity is thought to be the competitive inhibition versus Phe of Phe-dependent enzymes. Therefore, in addition, we investigated the effects of l-phenylalanine (Phe) and its influence on OTA toxicity in brain and neural retina cell cultures. Phe itself was found to differently affect brain and neural retina cell cultures. However, in both cultures OTA toxicity is not diminished by Phe. Therefore, our data indicate that at least in our cultures competition with Phe-dependent processes does not play a role in OTA toxicity.

Chouaniere D, Cassitto MG, Spurgeon A, Verdier A, Gilioli R. **An international questionnaire to explore neurotoxic symptoms.** Environ Res 1997;73(1-2):70-2.
BIOSIS COPYRIGHT: BIOL ABS. EURONEST (European Neurotoxic Solvents Toxicity) is a concerted action within the European Communities to use standardized methods available in all countries. The product of this effort is a new symptom questionnaire, EUROQUEST (European Questionnaire). The original version was developed in English. To obtain transcultural reliability, the translation of questionnaires into other languages must be validated. We adopted a procedure with translation, back translation, and agreement between translators. Ten other European countries also followed the same translation procedure for EUROQUEST.

Cowen T, Jenner C, Song GX, Santoso AW, Gavazzi I. **Responses of mature and aged sympathetic neurons to laminin and NGF: an in vitro study**. Neurochem Res 1997;22(8):1003-11.
CBAC COPYRIGHT: CHEM ABS While the potent effects of NGF and laminin on developing neurons are well documented, relatively little is known about the effects of, or altered availability of or altered responsiveness to, these substances on the growth of adult neurons. We have therefore examd. this question using explant cultures of sympathetic neurons from the superior cervical ganglion (SCG) of mature and aged rats. Explants were grown on substrata contg. different doses of laminin, either with or without added NGF in culture medium contg. fetal calf serum. Individually, laminin and NGF had relatively small effects on neurite outgrowth and length, which tended to be reduced in old neurons. In contrast, laminin in the presence of exogenous NGF exerted a powerful effect on nerve growth which was substantially greater than the sum of the effects of the individual factors. This synergy was evident in all exptl. groups and was greatest in old explants at high doses of laminin, where growth was comparable to that of mature neurons. The dose-response curve of old neurons to laminin in the presence of added NGF indicated reduced responsiveness. These results suggest that variations in the availability of laminin and/or exogenous NGF, together with altered patterns of neuronal responsiveness, may contribute to impaired neuronal plasticity in old age.

Deloye F, Doussau F, Poulain B. **[Mechanisms of botulinum and tetanus neurotoxins]**. Comptes Rendus Seances Soc Biol Filiales 1997;191(3):433-50. (Fre)
BIOSIS COPYRIGHT: BIOL ABS. Tetanus (TeNT) neurotoxin and botulinum (BoNT, serotypes A-G) neurotoxins are di-chain bacterial proteins of MWneurotoxins. They are the only causative agents of two severe neuroparalytic diseases, namely tetanus and botulism. The peripheral muscle spasms which characterise tetanus are due to a blockade of inhibitory (GABAergic and glycinergic) synapses in the central nervous system leading to a motor neurones disinhibition. In contrast, botulism symptoms are

only peripheral. They are consequent to a near irreversible and highly selective inhibition of acetylcholine release at the motor nerve endings innervating skeletal muscles. During the past decade, the cellular and molecular modes of action of clostridial neurotoxins has been near completely elucidated. After a binding step of the neurotoxins to specific membrane acceptors located only on nerve terminals, BoNTs and TeNT are internalized into neurons. Inside their target neurones, the intracellularly active moiety (their fight chain) is translocated from the endosomal compartment to the cytosol. The neurotoxins' light chains are zinc-dependent endopeptidases which are specific for one among three synaptic proteins (VAMP/synaptobrevin, syntaxin or SNAP-25) implicated in neurotransmitter exocytosis. The presence of distinct targets for BoNTs and TeNT correlates well with the observed quantal alterations of neurotransmitter release which characterize certain toxin serotypes. In addition, evidence for a second, non-proteolytic, inhibitory mechanism of action has been provided recently. Most likely, this additional blocking action involves the activation of neurone transglutaminases. Due to their specific action on key proteins of the exocytosis apparatus, clostridial neurotoxins are now widely used as molecular tools to study exocytosis.

Easton RM, Deckwerth TL, Parsadanian AS, Johnson EM Jr. **Analysis of the mechanism of loss of trophic factor dependence associated with neuronal maturation: a phenotype indistinguishable from Bax deletion.** J Neurosci 1997;17(24):9656-66.
During development, sympathetic neurons are critically dependent on nerve growth factor (NGF) for survival. Neurons isolated from the superior cervical ganglia (SCG) of embryonic rodents and maintained for 1 week in vitro undergo programmed cell death in response to NGF deprivation. As the cells mature in vitro and in vivo, however, these neurons develop a resistance to NGF deprivation and become much less acutely dependent on NGF for survival. Using an in vitro model of neuronal maturation, we confirmed that SCG neurons maintained in culture for 3-4 weeks did not experience a dramatic loss in viability after NGF removal, yet they did undergo the initial biochemical and genetic changes elicited by NGF deprivation of young neurons. NGF deprivation of mature neurons produced rapid decreases in glucose uptake and protein and RNA synthesis rates, increased phosphorylation of c-Jun, and an increase in c-jun mRNA. Mature neurons, however, experienced a block in the cell death program before the final stages of the pathway activated in young neurons, which includes the induction of c-fos mRNA and characteristic apoptotic nuclear changes. This maturation-induced block was indistinguishable by these criteria from the block produced by Bax deficiency. Expression of Bax in mature neurons restored the apoptotic pathway, such that after NGF removal, Bax-overexpressing mature neurons resumed the apoptotic program, including the induction of c-Fos and passage through a caspase checkpoint. Thus, a block in the apoptotic program at or near the BAX checkpoint accounts for the decreased dependence of mature neurons on neurotrophic factor to maintain survival.

Feldman HS, Dvoskin S, Halldin MH, Ask AL, Doucette AM. **Comparative local anesthetic efficacy and pharmacokinetics of epidurally administered ropivacaine and bupivacaine in the sheep.** Reg Anesth 1997;22(5):451-60.
BACKGROUND AND OBJECTIVES: Ropivacaine is the S(-) propyl homolog of bupivacaine and mepivacaine. Studies in humans have confirmed the results of studies in laboratory animals that ropivacaine is a long-acting local anesthetic with an anesthetic profile similar to bupivacaine. Acute, intravenous systemic toxicity studies have been conducted in sheep and dogs. Local anesthetic efficacy

has been studied after epidural administration in the dog. This study was initiated to determine the local anesthetic efficacy and pharmacokinetics of ropivacaine and bupivacaine after epidural administration in an experimental sheep model and to evaluate the sheep model as a model of experimental epidural anesthesia. METHODS: Twelve adult nonpregnant ewes were prepared with chronically implanted lumbar epidural catheters and arterial lines. Each sheep received injections of 5.0 mL ropivacaine and bupivacaine (0.5% and 0.75%) in a blinded, random, cross-over fashion. Onset and duration of sensory and motor blockade were evaluated. Arterial blood samples were drawn for serum drug concentration determinations and pharmacokinetic analysis. RESULTS: Onset and duration of motor blockade were similar for comparable concentrations of both drugs. Both concentrations of ropivacaine and bupivacaine 0.5% demonstrated differential neural blockade. The peak serum concentrations generally occurred within 8 minutes after administration. The terminal elimination half-life in serum was about 3.5-4.0 hours and 6 hours for ropivacaine and bupivacaine, respectively. No signs of systemic toxicity were observed. Results of sensory and motor blockade were consistent with previous studies in laboratory animals and humans. CONCLUSIONS: Ropivacaine produces sensory and motor blockade which is similar to that produced by equal concentrations of bupivacaine after epidural administration in the sheep. Peak serum concentrations produced no signs of systemic toxicity. The results of this study are consistent with previously published data from studies in laboratory animals and humans. The sheep model of experimental epidural anesthesia appears to be a clinically relevant method to evaluate experimental local anesthetics.

Gilbert ME. **Towards the development of a biologically based dose-response model of lead neurotoxicity.** Am Zool 1997;37(4):389-98.
BIOSIS COPYRIGHT: BIOL ABS. Biologically-based dose-response (BBDR) paradigms incorporate mechanistic toxicological data in the derivation of quantitative models to estimate risk. Developmental lead (Pb) exposure has been long associated with deficits in intellectual ability. To date, direct estimates of toxicant-induced functional alterations in brain that may underlie cognition have been lacking, obviating the utilization of quantitative modeling for toxicological endpoints of higher brain function. The utility of the long-term potentiation (LTP) model of synaptic plasticity in the context of Pb-induced cognitive deficits is explored in the present paper. In reviewing physiological and biochemical requirements of LTP that may overlap with cellular mechanisms of Pb toxicity, a neurobiological schema is constructed upon which we can begin to explore the possibility of applying BBDR models in neurotoxicology.

Greedon DJ, Tansey MG, Baloh RH, Osborne PA, Lampe PA, Fahrner TJ, Heuckeroth RO, Milbrandt J, Johnson EM Jr. **Neurturin shares receptors and signal transduction pathways with glial cell line-derived neurotrophic factor in sympathetic neurons**. Proc Natl Acad Sci U S A 1997;94(13):7018-23.
CBAC COPYRIGHT: CHEM ABS Neurturin (NTN) is a neurotrophic factor that shares homol. with glial cell line-derived neurotrophic factor (GDNF). Recently, a receptor complex has been identified for GDNF that includes the Ret tyrosine kinase receptor and a glycosylphosphatidylinositol-linked protein termed GDNFRalpha.. However, differences in the phenotype of Ret and GDNF knockout animals suggest that Ret has at least one addnl. ligand. The authors demonstrate that NTN induces Ret phosphorylation in primary cultures of rat superior cervical ganglion (SCG) neurons. NTN also caused

Ret phosphorylation in fibroblasts that were transfected stably with Ret and GDNFRalpha but not in cells expressing Ret alone. A glycosylphosphatidylinositol-linked protein also was important for NTN and GDNF signaling in SCG neurons; phosphatidylinositol-specific phospholipase C treatment of SCG cultures reduced the ability of NTN to phosphorylate Ret and the ability of NTN or GDNF to activate the mitogen-activated protein kinase pathway. NTN and GDNF also caused sustained activation of Ret and the mitogen-activated protein kinase pathway in SCG neurons. Finally, both NTN and GDNF activated the phosphatidylinositol 3-kinase pathway in SCG neurons, which may be important for the ability of NTN and GDNF to promote neuronal survival. These data indicate that NTN is a physiol. relevant ligand for the Ret receptor and suggest that NTN may have a crit. role in the development of many neuronal populations.

Guale FG, Burrows GE. **Evaluation of chick embryo spinal motoneuron cultures for the study of neurotoxicity.** Natural Toxins 1997;5(3):115-20.
BIOSIS COPYRIGHT: BIOL ABS. The objective of this study was to assess the performance of chick embryo motoneuron cultures for the study of neurotoxicants. The response of motoneurons to the cytotoxic effects of picrotoxin, strychnine, bulbocapnine, and the naturally occurring excitatory aminoacids, N-methyl D-aspartate (NMDA) and L-glutamate was studied by using a colorimetric viability assay using a vital dye. Selective cellular responses other than cell death were evaluated using a spectrofluorometric assay based on the response of an electrochromic styryl dye (Di-4-Anneps) to determine the expression of receptors for glycine, gamma-aminobutyric acid (GABA). NMDA, and L-glutamate by motoneurons in culture. The performance of chick embryo motoneurons (E7) in culture was useful and informative in neurotoxicologic studies. Motoneurons (E7) were found to express receptors for glycine, GABA, NMDA, and dopamine. The presence of the receptors and the inherent characteristics of motoneurons to generate action potential at an early embryonic stage (E4) makes this culture system a reliable model to conduct mechanistic studies as well as for predictive screening tests for agents of pharmacologic and toxicologic potential.

Hajimohammadreza I, Raser KJ, Nath R, Nadimpalli R, Scott M, Wang KK. **Neuronal nitric oxide synthase and calmodulin-dependent protein kinase IIalpha undergo neurotoxin-induced proteolysis.** J Neurochem 1997;69(3):1006-13.
Calpain (calcium-activated neutral protease) has been implicated as playing a role of neuronal injury in cerebral ischemia and excitotoxicity. Here we report that, in addition to extreme excitotoxic conditions [N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate challenges], other neurotoxins such as maitotoxin, A23187, and okadaic acid also induce calpain activation, as detected by m-calpain autolytic fragmentation and nonerythroid alpha-spectrin breakdown. Under the same conditions, calmodulin-dependent protein kinase II-alpha (CaMPK-IIalpha) and neuronal nitric oxide synthase (nNOS) are both proteolytically cleaved by calpain. Such fragmentation can be reduced by calpain inhibitors (acetyl-Leu-Leu-Nle-CHO and PD151746). In vitro digestion of protein extract from cortical cultures with purified mu- and m-calpain produced fragmentation patterns for CaMPK-IIalpha and nNOS similar to those produced in situ. Also, several other calpain-sensitive calmodulin-binding proteins (plasma membrane calcium pump, microtubule-associated protein 2, and calcineurin A) and protein kinase C-alpha are also degraded in neurotoxin-treated cultures. Lastly, in a rat pup model of acute excitotoxicity, intrastriatal injection of NMDA

resulted in breakdown of CaMPK-IIalpha and nNOS. The degradation of CaMPK-IIalpha, nNOS, and other endogenous calpain substrates may contribute to the neuronal injury associated with various neurotoxins.

Johnson SM, Luo X, Bywood PT. **Neurotoxic effects of kainic acid on substantia nigra neurons in rat brain slices.** Exp Neurol 1997;146(2):546-52.
Excitatory amino acids (EAAs) have been implicated as mediators of cell death in neurodegenerative diseases involving catecholamine neurons. Few studies, however, have examined the toxic effects of EAAs on identified catecholamine neurons in vitro. We have investigated the neurotoxic effects of kainic acid in a rat brain substantia nigra (SN) slice preparation. Rats (60-80 g) were anesthetised with halothane and killed by cervical dislocation. SN slices, 300 microm thick, were incubated at 35 degrees C in a modified Krebs solution in the presence or absence of kainic acid and then fixed and processed for either immunohistochemistry (IHC) or electron microscopy (EM). In IHC experiments, SN neurons were labeled using antibody to tyrosine hydroxylase (TH) coupled to diaminobenzidine. In control slices, the antibody labeled not only the cell body but also the prolific dendritic arbor of SN neurons. Treatment with 50 microM kainic acid for 15 min or 2 h resulted in loss of TH staining and apparent fragmentation of the dendrites. EM provided ultrastructural evidence for kainic acid-induced degeneration of the dendritic arbor of SN neurons. Typically, the dendritic membrane was broken, or diffuse and collapsed. Ultrastructural damage, including clumping and marginalization of chromatin and vacuolation of the cytoplasm, was also observed in cell bodies. Damage to the dendritic arbor may occur early in the neurotoxic events leading to cell death, preceding the loss of the cell body. Our observations are consistent with the postulated role of EAAs as mediators of catecholamine neuron death.

Kawakami J, Yamamoto K, Shimokawa M, Sawada Y, Asanuma A, Yanagisawa K, Iga T. **Neurotoxic study of H2 antagonists using Xenopus oocytes injected with mouse-brain mRNA.** Biol Pharm Bull 1997;20(9):1030-2.
To clarify the dominant mechanism for the convulsant activity of H2 antagonists, the effects of an H2 antagonist, cimetidine, on membrane currents induced by various agonists were investigated. In Xenopus oocytes injected with mouse-brain mRNA, acetylcholine (ACh), serotonin (5-HT), gamma-aminobutyric acid (GABA), glycine (Gly), glutamic acid (Glu), kainic acid (KA), quisqualic acid (QA) and N-methyl-D-aspartic acid (NMDA)-induced current responses were recorded under a voltage-clamp condition. Cimetidine inhibited GABA-induced currents in a concentration-dependent manner; however, the current responses induced by the other agonists were not modified. The IC50 of various H2 antagonists, famotidine, nizatidine, cimetidine and ranitidine, for GABA (10 microM)-induced current response were 66, 260, 450 and 980 microM, respectively. However, these values of cimetidine and ranitidine were 40-400 times higher than the reported brain and cerebrospinal fluid (CSF) concentration of H2 antagonists at the occurrence of a clonic convulsion in vivo. In conclusion, we observed an inhibitory effect of H2 antagonists on the GABA response; however, this inhibition of GABA-mediated neurotransmission may not be the dominant mechanism for H2 antagonist-induced clonic convulsion in vivo.

Kodavanti P Rs, Tilson HA. **Structure-activity relationships of potentially neurotoxic PCB congeners in the rat.** Neurotoxicology 1997;18(2):425-41.

BIOSIS COPYRIGHT: BIOL ABS. We have explored the effects of PCBs on Ca2+-homeostasis and inositol phosphates in an attempt to understand cellular mechanism(s) for neurotoxicity of PCBs. The selected prototypic congeners have non-dioxin-like (2,2'-dichlorobiphenyl; 2,2'-DCB; IUPAC #4; ortho-substituted) and dioxin-like (3,3',4,4'5-pentachlorobiphenyl; 3,3',4,4',5-PeCB; IUPAC #126; non-ortho substituted) properties. The hypothesis is that some PCBs in vitro alter Ca2+-homeostasis and interfere with intracellular second messengers. One of the consequences of this perturbation is protein kinase C (PKC) translocation, and these events could lead to cytotoxicity. Our results indicate that the non-dioxin like PCB (ortho-substituted one) is active in vitro and perturbed signal transduction mechanisms including Ca2+-homeostasis and PKC translocation. The effects were seen at relatively low concentrations (5-50 muM), whereas higher concentrations (>200 muM) were required to produce cytotoxicity. Results from SAR, in general, indicate that congeners with chlorine substitutions at ortho-position or low lateral substitutions (mostly meta-) are active in vitro where as non-ortho congeners are inactive. In summary, these results indicate that low lateral substitution (especially without para-substitution that favor coplanarity) or high lateral content in the presence of ortho-substitution (to hinder coplanarity) may be the most critical structural requirement underlying the activity of PCB congeners in vitro. Additional experiments with polychlorinated diphenyl ethers (PCDEs) and their analogs, where coplanarity is difficult regardless of degree and pattern of chlorination, provided important information supporting our hypothesis that coplanarity plays a key role in the activity of PCBs in vitro. For example, a PCB congener with 3,3',4,4'-chlorine substitutions is not active whereas a PCDE with the same chlorine substitutions is active. Similarly, 4,4'-DCB is not active whereas PCDE with 4,4'-substitutions is active. One major structural difference in PCDE when compared to the corresponding PCB is non-coplanarity. The PCBs compared here are coplanar and not active, whereas PCDEs are non-coplanar and active in vitro in neuronal preparations. Molecular mechanics calculations and conformational searches confirmed the extent of coplanarity among PCBs and PCDEs. Non-ortho PCBs are more coplanar in nature when compared to ortho-PCBs and PCDEs. These results demonstrate that the extent of coplanarity of certain chlorinated aromatic hydrocarbons can affect their potency in vitro, and ortho-substitutions on the biphenyl, which increase non-coplanarity, are characteristic of the most active PCB congeners.

Krum JM, Kenyon KL, Rosenstein JM. **Expression of blood-brain barrier characteristics following neuronal loss and astroglial damage after administration of anti-Thy-1 immunotoxin.** Exp Neurol 1997;146(1):33-45.
BIOSIS COPYRIGHT: BIOL ABS. In most regions of the CNS, vascular endothelial cells play an important role in maintaining the composition of the neuronal microenvironment by virtue of their blood-brain barrier (BBB) characteristics. The maintenance of the endothelial BBB phenotype in vitro has been attributed primarily to astrocytes but little attention has been paid the potential role of neurons. In this study we have attempted to injure or destroy neurons and fibers of passage in a circumscribed area while leaving vascular and glial elements intact in order to determine if neurons are involved in BBB maintenance in situ. The immunotoxin OX7-SAP, a conjugate of the Thy-1 antibody OX7 and the ribosome-inactivating protein saporin, was injected into the adult rat striatum to effect neuronal death at the injection site. Although neurons and fibers of passage were destroyed within the lesion, glial cells unexpectedly were also severely injured as determined by immunohistochemical expression of several neuronal and astroglial marker proteins and ultrastructural analysis. The microvasculature remained

intact, allowing a qualitative immunohistochemical analysis of several BBB markers at time points ranging from 3 to 28 days postinjection. Despite the loss of both neurons and astroglia within the lesions, the microvasculature continued to express the brain-type endothelial glucose transporter GLUT-1 at all time points examined. In contrast, the barrier to endogenous protein (rat serum albumin) and the expression of endothelial barrier antigen (EBA) decreased initially but recovered even in areas that contained minimal numbers of astroglia and neuronal elements. We conclude that intact neuronal or glial cells do not appear to be necessary for the maintenance in situ of the BBB properties examined herein.

Lacy DB, Stevens RC. **Recombinant expression and purification of the botulinum neurotoxin type A translocation domain.** Protein Exp Purif 1997;11(2):195-200.
BIOSIS COPYRIGHT: BIOL ABS. Botulinum neurotoxin type A in its fully activated form exists as a dichain protein consisting of a 50-kDa light chain and a 100-kDa heavy chain linked by a disulfide bond (B. R. DasGupta and H. Sugiyama, Biochem. Biophys. Res. Commun. 48, 108-112, 1972). The protein can be further subdivided into three functional domains: a catalytic domain corresponding to the light chain, a translocation domain associated with the N-terminal half of the heavy chain, and a binding domain as the C-terminal half. To facilitate further structural and functional studies on the mechanism of toxin translocation, we report here the recombinant Escherichia coli expression and purification of the isolated translocation domain with a yield of 1 mg pure protein per 1 g cell paste. Circular dichroism, enzyme-linked immunosorbent assays, and preliminary crystallization experiments verify proper protein folding. This reagent should serve as a key tool in elucidating the mechanism of translocation and in determining how the catalytic domain, a large 50-kDa metalloprotease, is delivered to the cytosol.

Larm JA, Beart PM, Cheung NS. **Neurotoxin domoic acid produces cytotoxicity via kainate- and AMPA-sensitive receptors in cultured cortical neurones.** Neurochem Int 1997;31(5):677-82.
Domoic acid, a naturally occurring kainoid, has been responsible for several outbreaks of fatal poisoning after shellfish ingestion, and we examined its neurotoxic mechanism in cultured murine cortical neurones. Using observations of neuronal viability and morphology, exposure to domoic acid for 24 h was found to induce substantial concentration-dependent neuronal cell death. Domoic acid-mediated neuronal death was attenuated by the non-N-methyl-D-aspartate receptor antagonist 6-cyano-7-nitro-quinoxaline-2,3-dione and the alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptor-selective antagonist LY293558 ((3S,4aR,6R,8aR)-6-[2-(1H-tetrazol-5-yl)-ethyl]-1,2,3, 4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic acid), but unaffected by NS-102 (5-nitro-6,7,8,9-tetrahydrobenzo[g]indole-2, 3-dione-3-oxime)--a low-affinity kainate receptor antagonist. Domoic acid was equipotent with (S)-AMPA (EC50 values 3.8 and 3.4 microM respectively); however, (S)-AMPA induced only 50% cell death compared to > 80% cell death induced by domoic acid. Kainate also killed > 80% of cortical neurones; however, domoic acid was about 19 times more potent than kainate (EC50 75 microM). We show the potent neurotoxicity of domoic acid for the first time in a pure neuronal model and indicate that domoic acid acts via high-affinity AMPA- and kainate-sensitive glutamate receptors to produce excitotoxic cell death.

Lopachin RM, Lehning EJ. **The relevance of axonal swellings and atrophy to gamma-diketone neurotoxicity: a forum position paper.** Neurotoxicology 1997;18(1):7-22.
BIOSIS COPYRIGHT: BIOL ABS. Traditionally, gamma-diketone neuropathy is classified as a distal

axonopathy and has been characterized by giant axonal swellings in CNS and PNS tissues. These swellings contain neurofilamentous masses and are associated with thinning and retraction of the myelin sheath. it has been proposed that this axonopathy is caused by direct gamma-diketone modification of neurofilaments (NFs) involving pyrrolation of E-amino groups on NF lysyl residues and possibly secondary autoxidation of the pyrrole r with creation of covalent NF-NF crosslinks. Neurofilaments are thought to undergo chemical modification as they progress along the axonal axis and, eventually, accumulate at distal nodes of Ranvier where their proximodistal movement is impeded. Development of swelling presumably initiates axonal degeneration and subsequent functional deficits. However, other research suggests that axonal swellings are a non-specific effect related to subchronic gamma-diketone exposure. Such evidence draws into question the mechanistic relevance of these swellings. In contrast, research conducted over the past decade indicates axonal atrophy is a specific morphologic component of gamma-diketone neuropathy which might have both functional and mechanistic importance. In this overview, the potential neurotoxicological significance of both axonal swellings and atrophy are evaluated critically. Based on the evidence to be presented, we propose that axonal atrophy is the morphological consequence of the molecular mechanism of gamma-diketone neuropathy. Accordingly, several mechanistic scenarios related to the development of atrophy will be discussed. It is hoped that this Forum will stimulate scientific debate and initiate laboratory investigations which will either confirm or refute the involvement of axonal atrophy in gamma-diketone neurotoxicity. Investigating gamma-diketone atrophy might provide insight into the mechanism of other toxic axonopathies which are also associated with reduced ax caliber; e.g., acrylamide and carbon disulfide neuropathies.

Mook-Jung I, Joo I, Sohn S, Kwon HJ, Huh K, Jung MW. **Estrogen blocks neurotoxic effects of beta-amyloid (1-42) and induces neurite extension on B103 cells.** Neurosci Lett 1997;235(3):101-4.
Clinical studies have shown that estrogen replacement therapy is associated with reduced risk of Alzheimer's disease (AD). We tested whether or not estrogen blocks neurotoxic effects of beta-amyloid (1-42) (A beta1-42) on cultured B103 cells. A beta1-42 (1 microM) induced typical necrotic cell death, as revealed by light and electron microscopic examinations. Co-administration of estrogen not only blocked A beta1-42 toxicity to a large degree, but also enhanced neurite extension. Pretreatment with estrogen was even more effective in blocking A beta1-42 toxicity. When added 18 h after the beginning of A beta1-42 treatment, estrogen was still effective in halting the progress of cell death and enhancing neurite extension. The protection against A beta1-42-induced neuronal death by estrogen was unlikely due to a blockade of lipid peroxidation injury, since estrogen completely failed to attenuate ferrous chloride-induced cell death. These results demonstrate that estrogen blocks A beta1-42-induced neurotoxicity and enhances neurite extension on B103 cells, both of which may well be underlying mechanisms of beneficial effects of estrogen in AD.

Ooie T, Terasaki T, Suzuki H, Sugiyama Y. **Quantitative brain microdialysis study on the mechanism of quinolones distribution in the central nervous system**. Drug Metab Dispos 1997;25 (7):784-9.
CBAC COPYRIGHT: CHEM ABS Brain interstitial fluid (ISF) concns., which regulate the toxicodynamic effect of quinolone antimicrobial agents (quinolones) in the central nervous system, have been detd. for norfloxacin, ofloxacin, fleroxacin, and pefloxacin using a quant. brain microdialysis technique. Steady-state brain ISF concns. of the quinolones were 7-30 times lower than the unbound

serum concns. due to restricted distribution in the brain. Cerebrospinal fluid concns. of the quinolones were approx. twice as high as the brain ISF concns., except for norfloxacin. Thus, it seems that an active efflux transport system across the blood-brain barrier is responsible for maintaining brain ISF concns. lower than unbound serum concns. at steady-state. A good correlation was obsd. for norfloxacin, ofloxacin, fleroxacin, and pefloxacin between brain ISF concns. and total brain concns. Moreover, a relatively small difference was obsd. among the quinolones for the in vitro brain slice-to-medium concn. ratio, compared with an 11-fold difference in the in vivo brain-to-unbound serum concn. ratio after i.v. infusion. These results indicate that the different quinolones studied all exhibit similar apparent binding and/or uptake by brain parenchyma, with an av. brain ISF-to-total brain concn. ratio of 0.688.

Repetto G, Sanz P, Repetto M. **Effects of cobalt on mouse neuroblastoma cells cultured in vitro**. Toxicol In Vitro 1995,9(4):375-9.
The effects of cobalt at different cellular levels in-vitro were studied. Cells from the mouse neuroblastoma cell line Neuro-2a were exposed to cobalt-chloride (7646799) at concentrations ranging from 0.1 milligram/liter (mg/l) to 1,000mg/l for 24 hours. The following toxicity indicators were assessed: cell proliferation by quantification of total protein content; cytoplasmic membrane integrity to cytosolic lactate-dehydrogenase (LDH) leakage; lysosomal hexosaminidase (HEX) release; LDH activity; mitochondrial succinate-dehydrogenase activity (SDH); relative neutral-red uptake by lysosomes (RNRU); lysosomal HEX activity; and acetylcholinesterase activity. Cobalt was not very toxic to neuroblastoma cell proliferation, with a concentration of 200 micromolar causing 50% inhibition of proliferation. Cytoplasmic membrane permeability was not specifically increased, and LDH leakage occurred only at high concentrations. Cobalt was, however, lysosomotropic, causing HEX release. The effects on lysosomal function were studied with RNRU and showed stimulation at low concentrations and inhibition at high concentrations. Neural acetylcholinesterase activity was decreased after an initial stimulation at low concentrations. LDH and SDH intracellular activities were both stimulated at low concentrations, with mitochondrial SDH activity being the most sensitive marker studied. The authors conclude that the metabolic stimulatory effects induced by cobalt were more marked than changes in cytoplasmic and lysosomal membrane permeability.

Risinger C, Deitcher DL, Lundell I, Schwarz TL, Larhammar D. **Complex gene organization of synaptic protein SNAP-25 in Drosophila melanogaster**. Gene 1997;194(2):169-77.
CBAC COPYRIGHT: CHEM ABS The evolutionarily conserved protein SNAP-25 (synaptosome-assocd. protein 25 kDa) is a component of the protein complex involved in the docking and/or fusion of synaptic vesicles in nerve terminals. The SNAP-25 gene (Snap) in the fruit fly Drosophila melanogaster has a complex organization with 8 exons spanning >120 kb. The exon boundaries coincide with those of the chicken SNAP-25 gene. Only a single exon 5 was found in Drosophila, whereas human, rat, chicken, zebrafish, and goldfish have 2 alternatively spliced versions of this exon. In situ hybridization and immunocytochem. to whole mount embryos show that SNAP-25 mRNA and protein are detected in stage 14 and later developmental stages, and are mainly localized to the ventral nerve cord. Thus, Snap has an evolutionarily conserved and complex gene organization, and its onset of expression in Drosophila melanogaster correlates with a time in neuronal development when synapses begin to be formed and when other synapse-specific genes are switched on.

Saito S. **Effects of lysophosphatidic acid on primary cultured chick neurons.** Neurosci Lett 1997;229 (2):73-6.
Lysophosphatidic acid caused growth cone collapse in primary cultured chick neurons. This action was dose dependent and the potency was almost identical in three different neuron types, dorsal root ganglion neurons, retinal neurons, and sympathetic ganglion cells. Fifty percent of growth cones were collapsed by 10(-6) M lysophosphatidic acid. The growth cone collapse started within 2 min after lysophosphatidic acid exposure and no homologous desensitization was observed. However, this action was reversible and not toxic to the neurons. Suramin, known as an antagonist to lysophosphatidic acid, itself had growth cone collapsing activity against cultured primary neurons.

Sanz P, Flores IC, Soriano T, Repetto G, Repetto M. **In vitro quantitative structure-activity relationship assessment of pyrrole adducts production by gamma-diketone-forming neurotoxic solvents**. Toxicol In Vitro 1995;9(5):783-7.
Pyrrole adduct formation by gamma diketone metabolites of neurotoxic derivatives of n-hexane (110543) and n-heptane (142825) was investigated in an in-vitro assay. Ten solvents were assayed in the in-vitro system: 2-hexanone (591786), 3,4-dimethylhexane (583482), 2,5-hexanedione (110134), 3,4-dimethyl-2,5-hexanedione (25234791), 2-hexanol (626937), 2,5-hexanediol (2935446), 4-heptanone (123193), 5-methyl-3-heptanone (541855), 6-methyl-2,4-heptanedione (3002231) and 4-heptanol (589559). Each solvent was incubated with a purified microsomal fraction of liver from male albino-Wistar-rats pretreated with phenobarbital, and the kinetics of pyrrole adduct formation was measured spectrophotometrically. Pyrrole adducts were formed at varying rates, and the behavior of n-heptane and n-hexane derivatives was similar. The reaction was delayed when the derivatives contained hydroxyl groups, and formation was favored in the presence of methyl groups. In-vitro neurotoxicity indexes were calculated based on pyrrole adduct formation and were correlated with published in-vivo data. The authors conclude that there was a good correlation between neurotoxic potency in-vivo and quantitative production of adducts in-vitro with both n-hexane and n-heptane derivatives. The measurement of in-vitro pyrrole adduct formation can be used to provide a neurotoxicity index in screening tests for potentially neurotoxic solvents.

Seabrook GR, Bowery BJ, Heavens R, Brown N, Ford H, Sirinathsinghi DJ S, Borkowski JA, Hess JF, Strader CD, Hill RG. **Expression of B1 and B2 bradykinin receptor mRNA and their functional roles in sympathetic ganglia and sensory dorsal root ganglia neurons from wild-type and B2 receptor knockout mice**. Neuropharmacology 1997;36(7):1009-17.
CBAC COPYRIGHT: CHEM ABS Bradykinin has been implicated in nociception and inflammation. To examine the relative significance of B1 and B2 bradykinin receptor subtypes in sympathetic and sensory ganglia, the electrophysiol. effects of bradykinin analogs and the expression of receptor subtype mRNA were examd. in wild-type and B2 knockout mice from which the B2 receptor gene had been deleted. In wild-type mice the B2 receptor agonist bradykinin depolarized superior cervical ganglia (SCG) and activated inward currents in dorsal root ganglia (DRG) neurons. Responses to the B1 receptor agonist, [des-Arg10]-kallidin, were seen only in SCG that had been pretreated with interleukins and the peptidase inhibitor captopril, but not in DRG neurons. The up-regulation of responses to [des-Arg10]-kallidin and substance P were blocked by indomethacin and, thus, were dependent upon cyclo-oxygenase activity. The effects of bradykinin were abolished in SCG and DRG's from B2 knockout mice

and this was correlated with the absence of B2 receptor mRNA in ganglia from these animals. However, despite the presence of B1 receptor mRNA in interleukin treated SCG from B2 knockout mice, no depolarizing effects of the B1 receptor agonist [des-Arg10]-kallidin were obsd. The successful elimination of bradykinin responses and B2 mRNA in sympathetic and sensory ganglia from B2 knockout mice, confirms that B2 receptors are the predominant functional bradykinin receptor subtype in these tissues and that B1 receptor mRNA is expressed in both sympathetic and sensory ganglia from these animals.

Shankar L, Ravindranath V, Boyd MR, Vistica DT, Shankar SK. **Histological, histochemical and autoradiographic evidence of in vitro neurotoxic effects of the novel antitumor agent, 9-methoxy-N2-methylellipticinium acetate.** Neurotoxicology 1997;18(1):89-95.
9-Methoxy-N2-methylellipticinium acetate (MMEA) exhibits selective cytotoxicity towards glial-derived human brain tumor cell lines comprising the U.S. National Cancer Institute preclinical drug screen. Neurotoxic potential of MMEA has been demonstrated in an in vitro model employing sagittal slices of rat brain. Histochemical staining of rat brain slices for lactate dehydrogenase (LDH) activity revealed decreased staining intensity following incubation with increasing concentrations of MMEA (0.1-100 microM). Cytological evaluation of paraffin sections stained with Cresyl Fast Violet revealed neuronal damage delineated by cytoplasmic vacuolation, and distention and fraying of the plasma membrane. No glial or vascular pathology could be discerned. Autoradiography, following exposure to 14C-MMEA, revealed distinct labelling of the large neurons of the brain stem, neurons in the thalamus and pyramidal neurons of the hippocampus, indicating neuronal uptake of the drug.

Sheridan RE, Deshpande SS, Nicholson JD, Adler M. **Structural features of aminoquinolines necessary for antagonist activity against botulinum neurotoxin.** Toxicon 1997;35(9):1439-51.
Certain aminoquinoline antimalarial compounds, such as chloroquine, antagonize the paralytic actions of botulinum neurotoxins (BoNT). These studies have been extended to determine the critical structural groups necessary for synthetic aminoquinolines to have antagonist activity against BoNT. Isolated mouse hemidiaphragms were maintained at 36 degrees C and indirectly stimulated; the resulting isometric twitch tensions were recorded as a measure of synaptic function. The muscles were exposed to the test compounds before being treated with a challenge concentration of BoNT (typically 0.2 nM of serotype A). The time to onset of 50% muscle paralysis due to BoNT was used to assess quantitatively the efficacy of the test compounds, which were then ranked on the basis of the concentrations necessary to delay paralysis by a specified time increment. Of the compounds tested, those having a 7-chloro-4-aminoquinoline configuration, similar to chloroquine (or the structurally similar 6-chloro-9-amino acridine group in quinacrine), were most effective. Truncation of the alkyl-amino-alkyl group from chloroquine and conversion of the 4-amino nitrogen to a primary amine did not significantly alter its effectiveness as a BoNT antagonist. However, the 6-chloro- or 8-chloro- isomers of chloroquine were essentially ineffective. These results suggest that aminoquinolines antagonize the paralytic actions of BoNT through interaction with a selective, stereospecific site that is not well correlated with antimalarial activity.

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

(14):2453-68.
BIOSIS COPYRIGHT: BIOL ABS. A radically new approach for the discriminative determination of various neurotoxins has been developed. This novel biosensor combines a highly sensitive acetylcholinesterase (AChE) biosensor with immobilized organophosphate hydrolase (OPH). The value of the new concept was demonstrated by the discrimination between carbamate and organophosphorus pesticides. It was shown that the response of traditional AChE-based biosensor to mixed samples containing paraoxon and carbofuran was not simply additive, and the measured concentrations of these pesticides were very different from their real concentrations. This combined OPH/AChE system was able to improve the accuracy of the AChE-based biosensor and to uniquely distinguish paraoxon in mixed solutions containing carbofuran. The presented approach promises a new perspective for real world analyses and opens a new area of discriminative determination of various species in multicomponent solutions.

Suzuki Y, Shimizu H, Kim SU. **Induction of micronucleus in NSC19 motoneuron cell line by genotoxic chemicals.** Neurotoxicology 1997;18(2):325-30.
BIOSIS COPYRIGHT: BIOL ABS. Micronucleus is formed when chromosomal fragments behave independently of remaining chromosomes during the division of cells damaged by genotoxic agents, and the frequency of micronucleus is considered to reflect genotoxic damage to cells. In order to obtain a simple, fast and low-cost assay system to evaluate genotoxicity in the nervous system, we utilized the micronucleus test in a NSC19 mouse neuroblastomaitroquinoline-N-oxide (4NQO). There was a significant increase in number of micronuclei in NSC19 cells following 24 hr exposure to 4NQO. Two other genotoxic chemicals, cytosine beta-D-arabinofuranoside (AraC) and ethylmethanesulfate (EMS), similarly increased the frequency of micronucleated cells in a dose-dependent manner. The micronucleus test described in the present study is useful as a short-term genotoxicity evaluation system for cells of nervous system origin.

Takasawa K, Terasaki T, Suzuki H, Ooie T, Sugiyama Y. **Distributed model analysis of 3'-azido-3'-deoxythymidine and 2',3'-dideoxyinosine distribution in brain tissue and cerebrospinal fluid**. J Pharmacol Exp Ther 1997;282(3):1509-17.
CBAC COPYRIGHT: CHEM ABS The restricted distribution of 3'-azido-3'-deoxythymidine (AZT) and 2',3'-dideoxyinosine (DDI) in brain tissue and cerebrospinal fluid (CSF) has been analyzed using the distributed model. The distribution vol. of AZT and DDI in brain tissue (Vbr) was found to be 1.07 .+-. 0.09 and 0.727 .+-. 0.030 mL/g brain, resp., in an in vitro brain slice uptake study. The pharmacokinetic parameters were obtained by fitting the concn.-time profiles of AZT and DDI in brain tissue and CSF after i.v. or i.c.v. administration taking the value of Vbr, the CSF bulk flow rate (2.9 mul/min), and the surface area of the cerebroventricular ependyma (2.0 cm2), using a nonlinear least squares program combined with a fast inverse Laplace transform. The efflux transport clearance (PSBBB,eff) across the blood-brain barrier (BBB) and the sym. permeability clearance (PSBBB) across the BBB for AZT were calcd. as 179 and 10.3 mul/min/g brain, resp. The efflux transport clearance (PSCSF,eff) across the blood-cerebrospinal fluid barrier (BCSFB) and the sym. permeability clearance (PSCSF) across the BCSFB for AZT were calcd. as 227 and 28.3 mul/min/mL CSF, resp. For the distribution of DDI, the PSBBB,eff and PSBBB were 79.2 and 2.03 mul/min/g brain, resp., while the PSCSF,eff and PSCSF for DDI were 196 and 5.88 mul/min/mL CSF, resp. Based on simulation studies using the fitted parameters,

a significant degree of efflux transport across the BBB and BCSFB has been suggested to be responsible for the restricted distribution of AZT and DDI in brain tissue and CSF, resp.

Theophilidis G, Benaki M, Papadopoulou-Mourkidou E. **Neurotoxic action of six pyrethroid insecticides on the isolated sciatic nerve of a frog (Rana ridibunda).** Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 1997;118(1):97-103.
The neurotoxic action of six pyrethroid insecticides, four type II, (flucythrinate, deltamethrin, fenvalerate, fluvalinate) and two type I (cis- and trans-permethrin) was compared on the isolated sciatic nerve of frog. The nerve was exposed to pyrethroids for 30 min and action potentials were recorded for more than 45 hr after exposure. From the plots of the amplitude of the compound action potential vs time, it was possible to estimate, for each compound, the minimum effective concentration, the concentration which is required to reduce the amplitude of the compound action potential to 50% of its control value (mEC50). Flucythrinate was the most toxic compound, while toxicity decreased in the value: deltamethrin > fenvalerate > fluvalinate >> cis-permethrin > trans-permethrin. Low neurotoxicity of cis-permethrin and trans-permethrin (type I pyrethroids) was expected. The neurotoxicity of type I pyrethroids is mainly due to an action at the synapse, which are not present in the frog sciatic nerve preparation. The relative potencies of the four type II compounds agree with their acute toxicity estimated using the LD50.

Wang Xingmin, Meng Xiaoqi, Wang Chenghuai. **[Detection of Clostridium botulinum type B neurotoxin gene by polymerase chain reaction]**. Zhonghua Weishwuxue He Mianyixue Zazhi 1997;17 (5):389-93. (Chi)
BIOSIS COPYRIGHT: BIOL ABS. A polymerase chain reaction (PCR) method was developed for detection of Clostridium botulinum type B neurotoxin gene by using a pair of oligonucleotide primers which were designed from the nucleotide sequence of the light chain of type B neurotoxin gene to amplify a fragment of 253bp. Twenty two strains of Clostridium botulinum type B were all positive in PCR and mouse lethal test. Sensitivity of the PCR was determined by using Clostridium botulinum type B CMCC(B) 64352. The results showed that a clear amplified fragment could be obtained from only 60 organisms of Clostridium botulinum type B. The specificity of the PCR was determined by using 53 strains belonging to other types of Clostridium botulinum and other clostridial species. All strains except LCL001,one of Clostridium botulinum type A, were negative in the PCR. The molecular weight and the digestion pattern with restriction endonuclease of the amplified product of the strain LCL001 were all coincident with those of Clostridium botulinum type B. It could be regarded as a strain of Clostridium botulinum type A which contained unexpressed type B neurotoxin gene, or signed it type A(B). Therefore, it suggests that the PCR system is excellent for identification of Clostridium botulinum because of not only its specificity and sensitivity, but also the feasibility of finding out the silent type B neurotoxin gene in other types of Clostridium botulinum.

Zheng J, He X, Yang A, Liu C. **Kinetic properties of nicotinic receptors in cultured rat sympathetic neurons from superior cervical ganglia**. Zhongguo Yaoli Xuebao 1997;18(4):330-3.
CBAC COPYRIGHT: CHEM ABS The aim was to analyze the kinetic properties of the effect of nicotine on nicotinic acetylcholine receptors (nAChR) in cultured sympathetic neurons from neonatal rat superior cervical ganglia (SCG). The whole-cell recording method of the patch-clamp technique was

used to record the currents induced by different concns. of nicotine. The concn.-response of nicotine was fitted with the Clark equation. The Hill coeff. (1.097) was detd. by fitting the nicotine responses of neuronal nAChR with the Clark equation. The theor. values of the nicotine effect, calcd. using the Clark equation with H = 1, were basically identical with the recorded currents. Interaction of nicotine and nAChR in rat SCG fits a single binding site model.

OCULAR TOXICITY

Clang C M, Aleo MD. **Mechanistic analysis of S-(1,2-dichlorovinyl)-L-cysteine-induced cataractogenesis in vitro.** Toxicol Appl Pharmacol 1997;146(1):144-55.
BIOSIS COPYRIGHT: BIOL ABS. Chronic exposure to low concentrations of the nephrotoxic cysteine conjugate S-(1,2-dichlorovinyl)-L-cysteine (DCVC) causes cataracts in mice. This study explored mechanisms of DCVC-induced cataractogenesis using explanted lenses from male Sprague-Dawley rats. Lenses placed in organ culture were exposed to 2.5 muM-1 mM DCVC for 24 hr. DCVC caused concentration and time-dependent changes in biochemical markers of toxicity (lenticular adenosine 5'-triphosphate (ATP) content, mitochondrial reduction of the tetrazolium dye MTT, and glutathione (GSH) content) at concentrations :25 muM. Lens clarity was adversely affected at concentrations :50 muM. Within 24 hr, 1 mM DCVC altered lens ATP content (-77 : 2%), mitochondrial MTT reduction (-40 : 3%), and GSH content (-19 : -4%) (percent difference from controls, $p < 0.05$). ATP was the most sensitive index of DCVC exposure in this model, while lens weight was not altered. The role of lenticular DCVC metabolism was investigated using the beta-lyase inhibitor aminooxyacetic acid (AOA) and the flavin monooxygenase (FMO) inhibitor methimazole (MAZ). AOA (1 mM) provided nearly complete protection from changes in biochemical parameters and lens transparency caused by DCVC, while MAZ (1 mM) provided only partial protection. The mitochondrial Ca2+ uniport inhibitor ruthenium red (30 muM) and the poly(ADP ribosyl)transferase inhibitor 3-aminobenzamide (3 mM) were only partially protective, whereas adverse changes in lens transparency and biochemical markers were not prevented by an antioxidant (2 mM dithiothreitol) or nontoxic transport substrates (200 muM probenecid or 10 mM phenylalanine, S-benzyl-L-cysteine or para-aminohippuric acid). Calpain inhibitors E64d (100 muM) and calpain inhibitor II (1 mM) were ineffective in preventing opacity formation caused by DCVC. In a small separate study, DCVC toxicity to explanted lenses from cynomologus monkeys was also ameliorated by coincubation with AOA. These results indicate that opacity formation by DCVC in rodent and primate lenses in vitro is primarily mediated via lenticular beta-lyase metabolism of DCVC to a reactive metabolite. Metabolism of DCVC by FMO and perturbations in mitochondrial calcium (Ca2+) homeostasis and increased poly(ADP-ribosylation) of nuclear proteins may play a limited role in opacity formation in vitro. However, opacity formation does not appear to be the result of oxidative stress or calpain activation. DCVC toxicity to the lens was not blocked with competitive inhibitors of the amino acid and organic anion transporters of DCVC as is found in the kidney.

Cottin M, Zanvit A. **Fluorescein leakage test: a useful tool in ocular safety assessment.** Toxicol In Vitro 1997;11(4):399-405.
BIOSIS COPYRIGHT: BIOL ABS. The fluorescein leakage test (FLT) provides information on the effects of xenobiotics on the impermeability (gate function) of epithelial cell monolayers, and their

recovery after exposure. The aim of this study was to assess the validity of this test in the ocular safety assessment of surfactant-based products with various irritant potencies. Madin-Darby canine kidney cells were grown to confluency on microporous membranes and exposed for 15 min to increasing concentrations of test substances. Damage was evaluated by measuring the amount of Na-fluorescein that passed through the monolayer in 30 min, starting just after exposure. Recovery was assessed 4, 24, 48 and 72 hr later. For each sample and each time point, the amounts of test substance that produced 10% and 20% leakage (FL10 and FL20) compared with a cell-free control were calculated. For the 43 samples, FL20 values ranged from 0.65 to 1000 mg/ml. These values increased or decreased with time according to the substance. In particular, cell monolayers showed very different recoveries after exposure to anionic and cationic substances with similar initial FL20 values. These in vitro data correlated well with historical Draize in vivo test data (Spearman's rho > 0.90). The FLT is therefore useful as a complement to other in vitro methods for the ocular safety evaluation of cosmetics.

Earl LK, Dickens AD, Rowson MJ. **A critical analysis of the rabbit eye irritation test variability and its impact on the validation of alternative methods**. Toxicol In Vitro 1997;11(3):295-304.
CBAC COPYRIGHT: CHEM ABS A review and discussion with 24 refs. Detg. the validity of alternative methods as replacements for the rabbit eye irritation tests is a goal of the scientific, regulatory, and political communities and is being evaluated in several studies. The results from the Draize rabbit eye test are used as a std. against which to compare the performance of the in vitro methods. However, the quant. performance of the modern Draize eye test is unknown. This paper is a review of the findings of a previous study to est. the historical variability and a comparison with more contemporary data in order to est. the performance of the modern methods. The question of whether it is practical to obtain an accurate description of in vivo variability of the modern Draize test is considered by calcg. the size of the interlab. study that would be required to det. whether variability had changed since 1971. The impact that in vivo variability has on the validation of alternative methods is then discussed. The authors conclude that validation studies have a greater chance of success if the alternative methods are soundly based on mechanisms of toxicity operating in vivo, the Draize data are well defined with regard to their variability, the goals of the study are realistic, and the customers of the study are in broad agreement with the study design and the in vivo data used as the ref. test set.

Gilleron L, Coecke S, Sysmans M, Hansen E, Van Oproy S, Marzin D, Van Cauteren H, Vanparys P. **Evaluation of the HET-CAM-TSA method as an alternative to the Draize eye irritation test.** Toxicol In Vitro 1997;11(5):641-4.
BIOSIS COPYRIGHT: BIOL ABS. The Hen's Egg Test-Chorioallantoic Membrane (HET-CAM) method was modified in our laboratory by means of microscopic evaluation, a clear description of the three in vitro endpoints (haemorrhage, lysis and coagulation) and the use of a test substance applicator (TSA). A previous study on 46 chemicals demonstrated the usage of the HET-CAM-TSA assay as a screening test for eye irritancy. In order to extend our database and to come to a more reliable conclusion concerning the use of the HET-CAM-TSA method, a second set of 60 test substances was tested. The in vitro irritation scores (IS) were compared with the in vivo modified maximum average scores (MMAS) calculated 24 hr after instillation. The MMAS irritancy threshold was set at 15.0. The results were analysed according to the Cooper's parameters (specificity, sensitivity and concordance with the Draize test) and the Pearson's correlation coefficient. It was concluded that the HET-CAM-TSA test

was a valuable screening test. To compensate for the misclassifications generated, it was also concluded that the HET-CAM-TSA method should be considered as a part of a test battery, together with the Bovine Corneal Opacity-Permeability (BCOP) assay.

Herbert KL, Sivak JG. **In vitro measure of ocular damage and recovery from aromatics**. J Toxicol Cutan Ocul Toxicol 1996;15(4):331-41.
The validity of an in-vitro method for the determination of ocular toxicity was tested. The in-vitro technique involved measurement, using a scanning laser system, of the focal length characteristics of a cultured bovine lens exposed to test agents. The results obtained using this method were compared to those reported using the in-vivo Draize testing method. The ocular effects of toluene (108883), styrene (100425), and xylene (1330207) were examined in both systems. In-vitro exposure to all three test agents damaged the bovine lens. The effect with xylene was much more rapid than that with the other agents. All lenses recovered from the damage following removal of the test agents; most recovered to a level of optical performance comparable to that of unexposed control lenses. Data from studies using the in-vivo Draize assay demonstrated corneal swelling following exposure to each of the three agents studied but did not show a sufficient range in scores to differentiate between the irritancy potential of the three agents. Data on the time elapsed to damage the lens, and recovery time obtained from the in-vitro studies, suggested that xylene was more irritating than the other two agents, and that the damage induced by toluene was longer lasting. The authors conclude that the in-vitro ocular toxicity assay described merits further study as an in-vitro alternative to in-vivo ocular toxicity testing.

Imbert D, Cullander C. **Assessment of cornea viability by confocal laser scanning microscopy and MTT assay.** Cornea 1997;16(6):666-74.
PURPOSE: Determination of excised cornea viability is of interest for transplant-storage evaluation, but also for in vitro diffusion-study design and ocular-toxicity assessment. By using simultaneous vital staining by calcein AM (CAM) and ethidium homodimer-1 (EH-1), as live and dead probes, respectively, we developed a confocal laser scanning microscopy (CLSM) assay to determine epithelial and endothelial viability and estimate cornea thickness. METHODS: New Zealand White rabbit corneas were stored in phosphate-buffered saline (PBS) or Optisol at 4 degrees C or at room temperature. At various times, corneas were stained with an EH-1/CAM solution and observed, without further treatment, by CLSM. Storage effects on the cornea were also assessed by using an MTT assay. RESULTS: Stromal swelling, shedding of the upper epithelial layers, and severe endothelial damage were observed after 4 h in PBS at room temperature. After 8 h, lower epithelial cell death was observed, along with loss of endothelial structure. Corneas stored in similar conditions in Optisol were indistinguishable from controls. Storage in Optisol at 4 degrees C affected the superficial layers of the corneal epithelium similarly at both 7 and 14 days. Extensive epithelial shedding and wing-cell death were observed at 25 days, but the basal layer remained approximately 50% healthy. Significant endothelial cell loss was observed at 25 days. MTT results were consistent with CLSM data in the medium-term storage study only. CONCLUSIONS: This CAM/EH-1 CLSM fluorescence assay is a sensitive index of viability in cornea, and thus may prove useful in investigations in which maintenance of vital functions in different cell layers is critical.

Schneider AI, Maier-Reif K, Graeve T. **The use of an in vitro cornea for predicting ocular toxicity.**

In Vitro Toxicol 1997;10(3):309-18.
BIOSIS COPYRIGHT: BIOL ABS. This paper presents the development of an in vitro cornea model to assess ocular irritancy. The in vitro cornea is an organotypic culture composed of corneal stromal fibroblasts embedded in a matrix of collagen with an underlying layer of endothelial cells and covered by corneal epithelial cells. AR corneal cells were isolated from fetal pig. Pure cultures of the three different cell types were obtained using an improved technique for isolation and cultivation. Subcultures were passaged until the 30th generation without showing signs of senescence. For a cytotoxicity assay in vitro corneas were constituted in microtiter plates. The cytotoxic response of five test chemicals with a well-characterized eye irritancy was studied using a modified MTT-assay, which possess many advantages over the traditional procedure. The irritation data for all test chemicals yielded by our test assay correlated well with the Draize test classification.

Sivak JG, Herbert KL. **Optical damage and recovery of the in vitro bovine ocular lens for alcohols, surfactants, acetates, ketones, aromatics, and some consumer products: a review**. J Toxicol Cutaneous Ocul Toxicol 1997;16(3):173-87.
CBAC COPYRIGHT: CHEM ABS A review and discussion with 31 refs. This research represents an effort to use abattoir-supplied biol. tissue as a screening system to reduce the use of live animals in ocular irritancy testing for industries in the chem., pharmaceutical, and cosmetic areas. The approach uses an automated lens scanning monitor developed to study the optical performance (focal length and transmittance) of the cultured ocular lens as a toxicol. assay for chem. agents that are potentially hazardous to the eye. It involves comparing lens sensitivity to a variety of chem. solns. from lists documented by a variety of international research efforts. The work involves objective measurement of the effect of various substances on the transmittance and focal characteristics of long-term whole lens cultures using lenses obtained from abattoir-supplied eyes. The results are graded by chem. and compared, when possible, to reported Draize ocular irritancy scores, and the results produced by other suggested in vitro alternatives. This work reviews the results obtained using this approach to evaluate the ocular irritancy potential of a variety of alcs., surfactants, acetates, ketones, and aroms. Addnl. work involving commonly used consumer products (contact lens solns.) is also reviewed.

Spielmann H, Liebsch M, Moldenhauer F, Holzhutter HG, De Silva O. **Modern biostatistical methods for assessing in vitro/in vivo correlation of severely eye irritating chemicals in a validation study of in vitro alternatives to the Draize eye test**. Toxicol In Vitro 1995;9(4):549-56.
As part of a project to replace the Draize eye test, biostatistical methods were used to assess in-vitro/in-vivo correlation of severely eye irritating chemicals. Two tests, the hen's egg chorioallantoic membrane (HET CAM) test and the mouse-3T3 cell neutral-red uptake cytotoxicity assay (NRU), were evaluated. Testing results for 200 chemicals in the two in-vitro assays did not sufficiently allow identification of severely eye irritating chemicals. Since the scoring system used for the HET CAM assay had been derived empirically, it was investigated whether the use of modern biostatistical methods would improve the selection of predictive endpoints of the assay. Comparison of HET CAM data with adverse reactions observed in different tissues of the rabbit's eye showed that complex regression models were better for describing in-vitro/in-vivo correlations than simple linear models. Discriminant analysis revealed that among the nine endpoints routinely determined in the HET CAM test, coagulation was the only acceptable endpoint to classify severely irritating chemicals according to European Union regulations.

The reaction time of appearance of coagulation of a 10% solution was the best discriminating factor. Since only severely irritating chemicals induce coagulation of the CAM within 50 seconds, this factor can be used to classify severely irritating chemicals as such without further testing in-vivo. Stepwise discriminant analysis allowed an in-vitro testing strategy to be developed to identify severely irritating chemicals by combining coagulation data for the HET CAM assay with NRU cytotoxicity data. Validity of the model for future data sets was assessed by cross validation. The authors conclude that this stepwise approach provides acceptable sensitivity and specificity for evaluation of severely eye irritating chemicals.

PHARMACOKINETIC AND MECHANISTIC STUDIES

Abbas R, Fisher JW. **A physiologically based pharmacokinetic model for trichloroethylene and its metabolites, chloral hydrate, trichloroacetate, dichloroacetate, trichloroethanol, and trichloroethanol glucuronide in B6C3F1 mice.** Toxicol Appl Pharmacol 1997;147(1):15-30.
BIOSIS COPYRIGHT: BIOL ABS. A six-compartment physiologically based pharmacokinetic (PBPK) model for the B6C3F1 mouse was developed for trichloroethylene (TCE) and was linked with five metabolite submodels consisting of four compartments each. The PBPK model for TCE and the metabolite submodels described oral uptake and metabolism of TCE to chloral hydrate (CH). CH was further metabolized to trichloroethanol (TCOH) and trichloroacetic acid (TCA). TCA was excreted in urine and, to a lesser degree, metabolized to dichloroacetic acid (DCA). DCA was further metabolized. The majority of TCOH was glucuronidated (TCOG) and excreted in the urine and feces. TCOH was also excreted in urine or converted back to CH. Partition coefficient (PC) values for TCE were determined by vial equilibrium, and PC values for nonvolatile metabolites were determined by centrifugation. The largest PC values for TCE were the fat/blood (36.4) and the blood/air (15.9) values. Tissue/blood PC values for the water-soluble metabolites were low, with all PC values under 2.0. Mice were given bolus oral doses of 300, 600, 1200, and 2000 mg/kg TCE dissolved in corn oil. At various time points, mice were sacrificed, and blood, liver, lung, fat, and urine were collected and assayed for TCE and metabolites. The 1200 mg/kg dose group was used to calibrate the PBPK model for TCE and its metabolites. Urinary excretion rate constant values were 0.06/hr/kg for CH, 1.14/hr/kg for TCOH, 32.8/hr/kg for TCOG, and 1.55/hr/kg for TCA. A fecal excretion rate constant value for TCOG was 4.61/hr/kg. For oral bolus dosing of TCE with 300, 600, and 2000 mg/kg, model predictions of TCE and several metabolites were in general agreement with observations. This PBPK model for TCE and metabolites is the most comprehensive PBPK model constructed for P450-mediated metabolism of TCE in the B6C3F1 mouse.

Ahmad AH, Sharma LD. **Disposition kinetics of gentamicin in normal and febrile goats following single dose intramuscular administration**. Indian J Anim Sci 1997;67(5):381-3.
CBAC COPYRIGHT: CHEM ABS A study of the disposition kinetics of gentamicin was conducted in goats following single dose i.m. administration (5 mg/kg). The effect of fever (induced by E. coli endotoxin) was obsd. on absorption, distribution and elimination of gentamicin. Serum concns. of gentamicin were detectable up to 6 h in both groups of goats. Significant differences in serum concns. of gentamicin were not obsd. at any time after injection between 2 groups of animals. The data were adequately described by a one- compartment model both in normal and febrile goats. Elimination half-

life of 104.8 min in fertile goats was calcd. The vol. of distribution (Vd/F) was 192.5 mL/kg in febrile goats whereas in normal goats it was 158.0 mL/kg. Based on these pharmacokinetic parameters a dosage regimen for gentamicin was calcd.

Annaert WG, Becker B, Kistner U, Reth M, Jahn R. **Export of cellubrevin from the endoplasmic reticulum is controlled by BAP31.** J Cell Biol 1997;139(6):1397-410.
Cellubrevin is a ubiquitously expressed membrane protein that is localized to endosomes throughout the endocytotic pathway and functions in constitutive exocytosis. We report that cellubrevin binds with high specificity to BAP31, a representative of a highly conserved family of integral membrane proteins that has recently been discovered to be binding proteins of membrane immunoglobulins. The interaction between BAP31 and cellubrevin is sensitive to high ionic strength and appears to require the transmembrane regions of both proteins. No other proteins of liver membrane extracts copurified with BAP31 on immobilized recombinant cellubrevin, demonstrating that the interaction is specific. Synaptobrevin I bound to BAP31 with comparable affinity, whereas only weak binding was detectable with synaptobrevin II. Furthermore, a fraction of BAP31 and cellubrevin was complexed when each of them was quantitatively immunoprecipitated from detergent extracts of fibroblasts (BHK 21 cells). During purification of clathrin-coated vesicles or early endosomes, BAP31 did not cofractionate with cellubrevin. Rather, the protein was enriched in ER-containing fractions. When BHK cells were analyzed by immunocytochemistry, BAP31 did not overlap with cellubrevin, but rather colocalized with resident proteins of the ER. In addition, immunoreactive vesicles were clustered in a paranuclear region close to the microtubule organizing center, but different from the Golgi apparatus. When microtubules were depolymerized with nocodazole, this accumulation disappeared and BAP31 was confined to the ER. Truncation of the cytoplasmic tail of BAP31 prevented export of cellubrevin, but not of the transferrin receptor from the ER. We conclude that BAP31 represents a novel class of sorting proteins that controls anterograde transport of certain membrane proteins from the ER to the Golgi complex.

Ary K, Rona K, Renczes G, Gachalyi B, Riesz T, Grezal G, Es Klebovich I. **[Pharmacokinetic study of nerisopam and its N-acetyl metabolite in rats]**. Acta Pharm Hung 1997;67(2-3):59-63. (Hun)
CBAC COPYRIGHT: CHEM ABS Three doses of nerisopam were administered to rats during a pharmacokinetic study of nerisopam and plasma concns. of nerisopam and its N-acetyl metabolite were detd. in parallel by means of a validated SPE-HPLC method developed by the authors. The pharmacokinetics of nerisopam could be described by a two-compartment open model in rats; it was absorbed rapidly and could be measured in plasma for about 8 h. The peak plasma concn. of the N-acetyl metabolite was reached rapidly a little bit later than that of the parent compd., similarly to the human plasma, and it could be measured for about 12 h. The pharmacokinetics of the N-acetyl metabolite could be described by a one-compartment open model. The fast appearance of the metabolite and the Cmax and AUC0-.infin. values higher than those of nerisopam refer to an intensive first-pass metab. The AUC-dose curves indicate that supposingly the mechanism transforming the N-acetyl metabolites are not as fast as the acetylation.

Bang-Andersen B, Lenz SM, Skjaerbaek N, Soby KK, Hansen HO, Ebert B, Bogeso KP, Krogsgaard-Larsen P. **Heteroaryl analogs of AMPA. Synthesis and quantitative structure-activity relationships**. J Med Chem 1997;40(18):2831-42.

CBAC COPYRIGHT: CHEM ABS A no. of 3-isoxazolol bioisosteres of (S)-glutamic acid (Glu), in which the Me group of (RS)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) was replaced by different 5-membered heterocyclic rings, were synthesized. Comparative in vitro pharmacol. studies on this series of AMPA analogs were performed using receptor binding assays (IC50 values) and the electrophysiol. rat cortical slice model (EC50 values). None of these compds. showed detectable affinity for the N-methyl-D-aspartic acid subtype of Glu receptors. Some of the compds. were weak inhibitors of [3H]kainic acid binding. The inhibitory effects on [3H]AMPA binding and agonist potencies at AMPA receptors of the Glu 3-isoxazolol bioisosteres were strictly dependent on the structure, electrostatic potential, and Me substitution of the heterocyclic 5-substituent. Thus, while (RS)-2-Amino-3-[3-hydroxy-5-(thiazol-2-yl)isoxazol-4-yl]propionic Acid (IC50 = 0.094 muM; EC50 = 2.3 muM) was approx. equipotent with AMPA (IC50 = 0.023 muM; EC50 = 5.4 muM), (RS)-2-amino-3-[3-hydroxy-5-(1H-imidazol-2-yl)isoxazol-4-yl]propi- onic acid (IC50 = 48 muM; EC50 = 550 muM) was some 2 orders of magnitude weaker than AMPA, and (RS)-2-amino-3-[3-hydroxy-5-(1-methyl-1H-imidazol-2-yl)isoxazol-4- -yl]propionic acid (IC50 > 100 muM; EC50 > 1000 muM) was inactive. Furthermore, (RS)-2-amino-3-[3-hydroxy-5-(2-methyl-2H-tetrazol-5-yl)isoxazol-4- -yl]propionic acid (IC50 = 0.030 muM; EC50 = 0.92 muM) was more potent than AMPA, whereas its N-1 Me isomer, (RS)-2-amino-3-[3-hydroxy-5-(1-methyl-1H-tetrazol-5-yl)isoxazol-4- -yl]propionic acid (IC50 = 54 muM; EC50 > 1000 muM) was inactive as an AMPA agonist. A quant. structure-activity relationship (QSAR) anal. revealed a pos. correlation between receptor affinity, electrostatic potential near the nitrogen atom at the ortho position of the heterocyclic 5-substituent, and the rotational energy barrier around the bond connecting the two rings. We envisage that a hydrogen bond between the protonated amino group and an ortho-positioned heteroatom of the ring substituent at the 5-position stabilize receptor-active conformations of these AMPA analogs.

Birnbaum LS, Keller DA. **Metabolism and pharmacokinetics of 1,3-butadiene**. Toxicology 1996;113 (3):14-6.
A general overview of recent papers dealing with the metabolism and pharmacokinetics of 1,3-butadiene (106990) (BD) was presented. The papers had been presented at a symposium on the health risks of BD and isoprene (78795) held at Blaine, Washington from June 27 to June 29, 1995. The goals of the session on BD metabolism and pharmacodynamics were to investigate the quantitative aspects of species differences in BD metabolism and pharmacokinetics and how human metabolism and pharmacokinetics of BD might compare with those observed in rodent and in-vitro studies. The use of physiologically based pharmacokinetic (PBPK) models for extrapolating the results of rodent studies was also considered. PBPK models have been proving to be very useful for testing hypotheses about critical tissue concentrations of BD metabolites, butadiene-monoepoxide (930223) (BDO) and butadiene-diepoxide (1464535) (BDO2), and how they can be incorporated into the risk assessment process. The authors concluded that PBPK models will also be useful in resolving conflicting observations about interspecies differences in butadiene metabolism. It was further noted that the session has identified two important areas for future research: applying PBPK models to quantitatively predict species differences in BD02 levels in blood and tissues and determining human variability in the metabolism of BD, BDO, and BDO2.

Bouanchaud DH. **Streptogramins: from parenteral to oral**. Infect Dis Ther 1997;21:51-66.

CBAC COPYRIGHT: CHEM ABS A review with 31 refs. Streptogramins are complex mixts. of macrolactones, synthesized by Streptomyces spp. Until recently, the complex structure of streptogramins made it difficult to study their mechanism of action; it was not possible to prep. semisynthetic derivs. with improved pharmacokinetic properties nor to precisely det. the PK/PD parameters of these drugs in humans. Novel streptogramins consist of two well-defined components, which act synergically at the ribosomal level. RP 59500 (quinupristin/dalfopristin) is a new semisynthetic injectable streptogramin. RPR 106972 (RPR 13919/RPR 160950) is a cocryst. assocn., derived from a natural streptogramin; this drug is well absorbed by the oral route and might be considered as an oral partner for RP 59500. Both drugs have similar effectiveness (MIC50 .ltoreq. 2 mg/L) against multi-resistant Staphylococcus aureus, S. epidermidis, Streptococcus pneumoniae, many other streptococci, Enterococcus faecium, Legionella spp., Moraxella catarrhalis, and Mycoplasma spp. This activity has been confirmed with animal models of severe infections and has also been obsd. in human infections (Phase III and II resp. for quinupristin/ dalfopristin and RPR 106972). In vitro antibacterial activity is discussed also.

Bourguet D, Raymond M, Berrada S, Fournier D. **Interaction between acetylcholinesterase and choline acetyltransferase: an hypothesis to explain unusual toxicological responses.** Pestic Sci 1997;51(3):276-82.
BIOSIS COPYRIGHT: BIOL ABS. Organophosphorus and carbamate insecticides are thought to have only one target site, acetylcholinesterase (EC 3.1.1.7). When this enzyme is inhibited, the neurotransmitter acetylcholine is not metabolized and polarization of the post-synaptic membrane does not take place. But, what happens when the cholinesterase becomes resistant or when neurotransmitter levels are diminished? Here, we report results suggesting that choline acetyltransferase (EC 2.3.1.6), the enzyme responsible for the acetylcholine production, may be involved either as an alternative pesticide target site or as a factor enhancing survival during insecticide exposure. This underlines the concept that the pivotal step for insecticide toxicology is not the acetylcholinesterase activity but the amount of acetylcholine present. This latter can only fluctuate between an upper and a lower threshold, and crossing one of these two thresholds leads to the death of the insect. The interaction between acetylcholinesterase and choline acetyltransferase activities would explain the astonishing toxicological phenomenon that, in some conditions, mortality decreases when insecticide concentration increases.

Burstein AH, Gal P, Forrest A. **Evaluation of a sparse sampling strategy for determining vancomycin pharmacokinetics in preterm neonates: application of optimal sampling theory**. Ann Pharmacother 1997 Sep;31:980-3.
IPA COPYRIGHT: ASHP To use optimal sampling theory to determine the fewest vancomycin concentrations required and the appropriate sampling times to calculate vancomycin pharmacokinetic parameters in neonates, an unblinded evaluation in 11 neonates with presumed sepsis was conducted; 12 courses of intravenous (IV) vancomycin 20 mg/kg were administered and blood samples were collected 3 and 9 h after initiation of a 1 h infusion of the first dose. A 2 compartment model was fitted to vancomycin concentrations using iterative 2 stage analysis. Pharmacokinetic parameter estimates were used for determination of optimal sampling times for 2, 3, and 4-sample strategies with subsequent generation of 2, 3, and 4 sample concentration data for 100 cases. For estimation of total clearance and volume in the central and peripheral compartments, all strategies performed similarly with no difference in efficiency or bias and precision of estimates. It was concluded that for clinical evaluations 2

appropriately timed samples are adequate for estimation of vancomycin clearance in neonates.

Cao J, Wang D, Zhou S, Xie H, Qiu Y, Hao Y. **[Pharmacokinetics of norfloxacin nicotinate in pigs after intramuscular administration and in chickens after oral administration]**. Huazhong Nongye Daxue Xuebao 1997;16(4):361-6. (Chi)
CBAC COPYRIGHT: CHEM ABS Pharmacokinetic variables of a new quinolone antimicrobial, norfloxacin nicotinate, were studied in Hubei white pigs and chickens. The drug was given to 8 healthy pigs at a dosage of 10 mg per kg of body wt. i.m. and to 8 healthy chickens at a dosage of 10 mg per kg of body wt. orally. Plasma norfloxacin nicotinate concns. were detd. by using high performance liq. chromatog., with a limit of detection of 25 ng of norfloxacin nicotinate/mL of plasma. Plasma norfloxacin nicotinate pharmacokinetic variables were calcd. by using a two-compartment open model in pigs and a one-compartment open model in chickens. The dosage regiments of the drug for pigs and chickens were calcd. based on this study.

Chow HH. **A physiologically based pharmacokinetic model of zidovudine (AZT) in the mouse: model development and scale-up to humans.** J Pharm Sci 1997;86(11):1223-8.
After having been used in treating HIV infection for a decade, zidovudine (AZT) continues to be an essential component of antiretroviral regimens. Because antiviral responses and toxicity of AZT seem to be related to cells in specific target tissues, being able to understand and predict the distribution of AZT into different pharmacologically and toxicologically relevant tissues is therefore critically important to improving the efficacy and minimizing the toxicity of AZT therapy. This study was designed to develop a physiologically based pharmacokinetic model to help describe and predict the time course of AZT levels in different tissues. The model was developed in the mouse and then scaled up to predict human situations.

Clark JM. **Insecticides as tools in probing vital receptors and enzymes in excitable membranes.** Pestic Biochem Physiol 1997;57(3):235-54.
BIOSIS COPYRIGHT: BIOL ABS. In reflecting upon insecticides and their use, it is easy to dwell on their well-documented problems as environmental pollutants and nontarget toxicants. Yet very few chemical industries have been as responsive or as innovative as the agrochemical companies in providing society with new and novel compounds that have allowed us to produce and protect the high quality food and fiber necessary to feed and clothe an ever increasing human population. Given the effectiveness and wide-spread use of insecticides, it was naive not to have envisioned the environmental impacts that these chemicals have caused. Nevertheless, the impacts of insecticide use have resulted in a much more complete and rigorous understanding of the toxicokinetics and toxicodynamics of environmental contaminants, their environmental fate and degradative processes, and an increased insight on mechanisms leading to acquired drug tolerance and resistance. Such understanding would certainly not have been possible without the explicit structure-activity relationships provided by insecticidal chemicals. The availability of homologous series of insecticidal analogues, toxic and nontoxic insecticidal enantiomers, and radiolabeled insecticides have provided us with invaluable tools in the elucidation of how insecticides bind receptors and ultimately produce their toxic response. Pesticide science owes a debt of gratitude to the agrochemical industry for making these compounds readily available to environmental researchers and academic scientists alike. In this review, I examine

the mode of action studies of three structurally dissimilar insecticides on the nervous system. These insecticides are DDT, a chlorinated aromatic hydrocarbon that acts as a nerve toxin by modifying the kinetics of voltage-dependent ion channels associated with the neurolemma; deltamethrin, a synthetic pyrethrin analog that acts in a similar fashion as DDT; and azinphosmethyl, a phosphorodithioate organophosphate that acts as nerve toxin by competitively inhibiting acetylcholinesterase (Fig. 1). The activity of these insecticides are assessed using three functional assays: (i) the enhanced release of neurotransmitters from isolated presynaptic nerve terminals; (ii) the behavioral and mortality responses of the ciliate organism, Paramecium; and (iii) the inhibition of acetylcholinesterase associated with azinphosmethyl resistance in Colorado potato beetle. In each case, I summarize our findings in terms of the future benefit that they may provide.

Clewell HJ 3rd, Gentry PR, Gearhart JM. **Investigation of the potential impact of benchmark dose and pharmacokinetic modeling in noncancer risk assessment.** J Toxicol Environ Health 1997;52 (6):475-515.
There has been relatively little attention given to incorporating knowledge of mode of action or of dosimetry of active toxic chemical to target tissue sites in the calculation of noncancer exposure guidelines. One exception is the focus in the revised reference concentration (RfC) process on delivered dose adjustments for inhaled materials. The studies reported here attempt to continue in the spirit of the new RfC guidelines by incorporating both mechanistic and delivered dose information using a physiologically based pharmacokinetic (PBPK) model, along with quantitative dose-response information using the benchmark dose (BMD) method, into the noncancer risk assessment paradigm. Two examples of the use of PBPK and BMD techniques in noncancer risk assessment are described: methylene chloride, and trichloroethylene. Minimal risk levels (MRLs) based on PBPK analysis of these chemicals were generally similar to those based on the traditional process, but individual MRLs ranged from roughly 10-fold higher to more than 10-fold lower than existing MRLs that were not based on PBPK modeling. Only two MRLs were based on critical studies that presented adequate data for BMD modeling, and in these two cases the BMD models were unable to provide an acceptable fit to the overall dose-response of the data, even using pharmacokinetic dose metrics. A review of 10 additional chemicals indicated that data reporting in the toxicological literature is often inadequate to support BMD modeling. Three general observations regarding the use of PBPK and BMD modeling in noncancer risk assessment were noted. First, a full PBPK model may not be necessary to support a more accurate risk assessment; often only a simple pharmacokinetic description, or an understanding of basic pharmacokinetic principles, is needed. Second, pharmacokinetic and mode of action considerations are a crucial factor in conducting noncancer risk assessments that involve animal-to-human extrapolation. Third, to support the application of BMD modeling in noncancer risk assessment, reporting of toxicity results in the toxicological literature should include both means and standard deviations for each dose group in the case of quantitative endpoints, such as relative organ weights or testing scores, and should report the number of animals affected in the case of qualitative endpoints.

Cosson VF, Fuseau E, Efthymiopoulos C, Bye A. **Mixed effect modeling of sumatriptan pharmacokinetics during drug development. I: interspecies allometric scaling.** J Pharmacokinet Biopharm 1997;25(2):149-67.
Allometric scaling is an empirical examination of the relationships between the pharmacokinetic

parameters and size (usually body weight), but it can also involve brain weight for metabolized drug. Through all species, the protein binding of sumatriptan is similar (14-16%), and its metabolic pathway undergoes extensive oxidative deamination involving the monoamine oxidase A isoenzyme. These similarities across species suggested the possible relevance of an allometric analysis. Toxicokinetic data were collected from rats, pregnant rabbits, and dogs in animal pharmacokinetic studies where sumatriptan was administered intravenously to the animals at doses of 5 mg/kg. 0.25 mg/kg, and 1 mg/kg, respectively. Animal data were pooled and analyzed in one step using a mixed effect modeling (population) approach. The kinetic parameters predicted in any species were close to the observed values by species: 77 L/hr vs. 80 L/hr in man for total clearance, 137 L vs. 119 L for distribution volume at steady state. The value of the mixed effect modeling approach compared to the two-step method was demonstrated especially with the possibility of including covariates to describe the status of animal (e.g., pregnancy) in the model. Knowledge of the animal kinetics, dynamics, and metabolism of a drug contributes to optimal and expeditious development. Valuable information for the design of the first-dose-in-man study may emerge from more creative data analysis based on all the information collected during the preclinical and ongoing nonclinical evaluation of a new drug.

Dalla Costa T, Nolting A, Rand K, Derendorf H. **Pharmacokinetic-pharmacodynamic modeling of the in vitro anti infective effect of piperacillin-tazobactam combinations**. Int J Clin Pharmacol Ther 1997;35(10):426-33.
CBAC COPYRIGHT: CHEM ABS The aim of the study was to investigate the in vitro antiinfective effect of piperacillin-tazobactam (PIP-TZB) combinations on Escherichia coli in simulations of free concn. time profiles of both drugs, similar to those obtained in human tissue after i.v. bolus administrations. An in vitro diln. model was used to expose E. coli ATCC 35218 (beta-lactamase producer) to various piperacillin-tazobactam concn. profiles obtained after i.v. bolus multiple dose, using different dose ratio combinations (1: 4, 1: 8, 1: 16) and dosing regimens, ranging from once-a-day to 4 times a day. The antimicrobial effect was evaluated by detn. of the no. of bacteria over time. The concn. of PIP in the model was detd. by HPLC. A modified Emax model was used to describe the pharmacodynamic effect. The model was linked with the piperacillin concns. detd. exptl. to provide a pharmacokinetic-pharmacodynamic (PK-PD) model. The EC50 for piperacillin alone averaged 5.66 .+-. 0.29 mug/mL. The EC50 for all doses of piperacillin combined with 0.5 g of tazobactam were dose-dependent and averaged 1.70, 3.95, and 6.14 mug/mL for PIP 2, 4, and 8 g, resp. By increasing the dose of TZB in combination with a fixed dose of PIP, a decreased EC50 was obsd. The PK-PD model allowed a detailed evaluation of the dosing regimens investigated. The results suggested that for these combinations, 3 times a day administration is as effective as 4 times a day. Pharmacodynamic activity of the combinations can be prolonged by sufficiently high inhibitor concns.

Dejongh J, Blaauboer BJ. **Evaluation of in vitro-based simulations of toluene uptake and metabolism in rats.** Toxicol In Vitro 1997;11(5):485-9.
BIOSIS COPYRIGHT: BIOL ABS. The uptake of toluene in the rat from a closed exposure chamber was simulated with a physiologically based pharmacokinetic (PB-PK) model. Six different parameter sets for toluene biotransformation in vitro were subsequently substituted in the model while keeping all other model parameters constant. Simulations of toluene uptake and metabolism based on these six in vitro-derived biotransformation parameter sets were compared with two empirical in vivo data sets on

the decrease of toluene concentrations in closed exposure chambers. It was observed that simulations based on in vitro-derived biotransformation parameters gave similar or better results than simulations across these two in vivo data sets. It is concluded that the results from most studies on toluene biotransformation in vitro resulted in adequate simulations of uptake and metabolism of toluene in vivo. These results support earlier findings on application of in vitro techniques to derive parameters for PB-PK models.

Dejongh J, Blaauboer BJ. **Simulation of lindane kinetics in rats.** Toxicology 1997;122(1-2):1-9.
The kinetics of lindane were modelled in the male rat with a physiologically-based pharmacokinetic (PB-PK) model. The model was parameterized by using reference physiological parameter values and partition coefficients that were reported earlier in the literature. First order biotransformation and gastro-intestinal absorption constants for lindane were obtained by visually fitting the model to literature data on lindane disposition in vivo after a single oral dose. The model was validated by simulating the disposition of lindane in vivo after single intraperitoneal and chronic oral dosage and comparing simulated with experimental results. It was concluded that the present model can adequately simulate most of the reported data on lindane kinetics.

Demilo AB, Gelman DB, Bordas B. **Benzoylbiuret insect chitin inhibitors: structure-activity correlations derived from an in vitro clasper assay and an in vivo mosquito adult emergence assay**. J Entomol Sci 1997;32(2):212-28.
CBAC COPYRIGHT: CHEM ABS Chitin-synthesis inhibitory effects of nine 1-benzoyl-5-phenyl-biurets and six 1-benzoyl-5-phenyl-4-thiobiurets were detd. in an in vitro assay using male pharate adult claspers of the European corn borer, Ostrinia nubilalis (Hubner). Incorporation of labeled N-acetylglucosamine into the clasper tissue was effectively inhibited by the benzoylbiurets (IC50 values ranged from 0.032 to 8.2 ppm). QSAR of the benzoylbiurets were analyzed by linear regression anal. The anal. used yellowfever mosquito (Aedes aegypti (L.)) adult emergence data (LC50 values) and in vitro chitin-synthesis inhibition data (IC50 values) as biol. endpoints and a set of physicochem. parameters (independent variables) of the para substituent of the anilide moiety. Stepwise regression anal. of the in vivo data provided a significant four-parameter equation involving Hammett sigmap and Hansch-Fujita pi consts., and two indicator variables. Anal. of the in vitro chitin-synthesis inhibition data yielded a significant two-parameter equation incorporating sigmap and an indicator variable. In vivo larvicidal activity against A. aegypti and the house fly (Musca domestica L.) was enhanced by electron-withdrawing and lipophilic substituents in the para position of the anilide moiety. In contrast, in vitro chitin-synthesis inhibition was favored by electron-donating substituents. Thus, equations derived from in vivo activity data describe mainly pharmacokinetic processes such as transport and metab. Structural requirements for intrinsic activity of the benzoylbiurets at the putative receptor site were better represented by the equation derived from the in vitro chitin-synthesis inhibition data.

Derendorf H, Mollmann H, Hochhaus G, Meibohm B, Barth J. **Clinical PK/PD modeling as a tool in drug development of corticosteroids**. Int J Clin Pharmacol Ther 1997;35(10):481-8.
CBAC COPYRIGHT: CHEM ABS Corticosteroids are used for the treatment of a variety of different diseases both locally and systemically. Most therapeutic effects result from glucocorticoid receptor-mediated events, and there seems to be no substance-specific difference in the post-receptor reaction

cascade. Therefore, the extent and duration of glucocorticoid effects depend only on the availability of the resp. steroid at the receptor site and its affinity to the receptor. This makes glucocorticoids an ideal candidate for PK/PD modeling. Availability at the receptor site is governed by pharmacokinetic parameters such as bioavailability, clearance, protein binding, and vol. of distribution. The receptor affinity can easily be measured in vitro. A suitable indirect-response PK/PD model is presented that allows description of the receptor-mediated drug effects such as endogenous cortisol suppression as a function of time. Furthermore, this model allows prediction of the systemic activity of newly developed corticosteroids based on their pharmacokinetics and their resp. receptor-binding affinity. The model can also be applied in order to study systemic steroid effects after topical administration or to investigate the effect of the time of dosing on cortisol suppression. Comparison of predictions based on this model and results from large clin. studies are in excellent agreement. Corticosteroids may represent an ideal class of drugs for the successful use of PK/PD modeling during drug development allowing to save time and expenses.

Fasciano J, Hatzidimitriou G, Yuan J, Katz JL, Ricaurte GA. **N-methylation dissociates methamphetamine's neurotoxic and behavioral pharmacologic effects.** Brain Res 1997;771(1):115-20.
The present studies further examined the effect of N-methylation on the behavioral and neurotoxic effects of methamphetamine. Drug discrimination studies employing a training dose of 1 mg/kg of methamphetamine were used to confirm and extend previous behavioral studies indicating that N-methylation reduced the behavioral activity of methamphetamine 5- to 10-fold. In subsequent neurotoxicity studies, rats received doses of methamphetamine (10 mg/kg, s.c., every 6 h x 5) or its N-methylated derivative, N,N-dimethylamphetamine (100 mg/kg, s.c., every 6 h x 5) that, based on the results of the behavioral studies, would be expected to produce behaviorally equivalent effects. Saline-treated rats served as controls. Two weeks after treatment, the status of brain dopamine (DA) and serotonin (5-HT) neurons was assessed by measuring DA and 5-HT axon terminal markers. As anticipated, methamphetamine produced neurochemical deficits indicative of DA and 5-HT axon terminal damage. By contrast, despite the fact that it was given at a dose behaviorally equivalent to methamphetamine, N-N-dimethylamphetamine failed to produce signs of DA or 5-HT neurotoxicity. These results indicate that N-methylation dissociates methamphetamine's neurotoxic and behavioral pharmacologic effects, and suggest that it may be possible to separate the neurotoxic and pharmacologic effects of other substituted amphetamine derivatives with potentially useful clinical activity (e.g. fenfluramine and methylenedioxymethamphetamine).

Ferreira IL, Duarte CB, Carvalho AP. **'Chemical ischemia' in cultured retina cells: the role of excitatory amino acid receptors and of energy levels on cell death.** Brain Res 1997;768(1-2):157-66.
In this study, we determined whether the retina cell death observed in response to an ischemic-like insult is related to an overactivation of the ionotropic glutamate receptors and/or to a collapse of the energy levels. Cultured chick retina cells were submitted to 'chemical ischemia' by metabolic inhibition with sodium cyanide and iodoacetic acid, which block oxidative phosphorylation and glycolysis, respectively. The assessment of neuronal injury was made spectrophotometrically by quantification of cellularly reduced MTT, which gives information about mitochondrial function, or by staining with fluorescein diacetate (FDA), which correlates with changes in the plasma membrane permeability. 'Chemical

ischemia' induced both an acute and a delayed time-dependent degeneration of chick retina cells. We observed that 2 min after the ischemic insult, the levels of ATP were reduced to a minimum. On the other hand, the metabolic inhibition induced the release of aspartate, glutamate and gamma-aminobutyric acid, and the activation of AMPA/kainate receptors during the period of metabolic arrest was partially responsible for the loss of mitochondrial function. However, the NMDA and non-NMDA receptor antagonists (MK-801 and CNQX) did not prevent the plasma membrane damage caused by sodium cyanide and iodoacetic acid. The results show that the collapse of the energy levels, rather than the increase in excitatory amino acids, appears to underlie the observed cell injury, suggesting an important relationship between ischemia-induced depletion of high-energy metabolites and retina cell degeneration.

Frazier JM. **Predictive toxicodynamics: empirical/mechanistic approaches.** Toxicol In Vitro 1997;11 (5):465-72.
BIOSIS COPYRIGHT: BIOL ABS. A major objective of the toxicological sciences is to predict the in vivo toxicological consequences of human exposure to pure chemicals, complex mixtures and commercial formulations. Historically, the experimental approach to this goal has been to investigate toxicological processes in whole animal models and extrapolate the results obtained to predict human risk using various extrapolation procedures (high-dose/low-dose extrapolation, interspecies extrapolation and route-to-route extrapolation). Can in vitro methods be more widely employed in quantitative risk assessment? One major limitation to the broader application of in vitro toxicity testing methods is the lack of validated techniques for the extrapolation of in vitro-derived toxicodynamic data to the in vivo situation. The objective of this paper is to describe some approaches to the development of techniques to extrapolate in vitro toxicity testing data to predict in vivo toxicological responses. An empirical approach within the context of a mechanistic framework is explored. The basic hypothesis is that the in vivo response can be constructed from a cellular toxicity factor that accounts for the cellular response and a toxicodynamic factor that relates toxicological events at the cellular level to the observable in vivo responses. A predictive paradigm to describe the in vivo acute target organ toxicity (hepatotoxicity) of a model chemical (cadmium) is discussed. The cellular toxicity factor is derived from in vitro toxicity testing studies using isolated rat. hepatocytes. The toxicodynamic factor is derived through Biologically-Based Response (BBR) modelling techniques to predict target organ toxicity markers (i.e. plasma hepatic enzyme levels as markers for acute hepatotoxicity). The ultimate goal is to develop validated extrapolation procedures that can be applied to predicting target organ toxicity quantitatively in human populations based on in vitro toxicity studies using human cellular models.

Fujii H, Koscielniak J, Berliner LJ. **Determination and characterization of nitric oxide generation in mice by in vivo L-Band EPR spectroscopy.** Magn Reson Med 1997;38(4):565-8.
The authors have shown direct, real-time, in vivo measurement of nitric oxide (NO) in mice by using the water soluble metal chelator complex, N-methyl-D-glucamine dithiocarbamate (MGD), and Fe(II) as monitored by EPR at L-band. The three-line EPR spectrum from the product [(MGD)2-Fe(II)-NO] was observed noninvasively in lipopolysaccharide (LPS)-treated mice. The spectrum was markedly suppressed by the administration, before LPS injection, of phenyl N-tert-butyl nitrone (PBN), an inhibitor of the expression of induced nitric oxide synthase (iNOS). When 15N-arginine was administered to LPS-treated mice, a diagnostic EPR spectrum was observed, consisting of both three-

and two-line EPR signals, due to (MGD)2-Fe(II)-14NO and (MGD)2-Fe(II)-15NO, respectively. The results strongly suggested that the NO detected in these experiments was synthesized by iNOS. In vivo EPR measurements of [(MGD)2-Fe(II)-NO] at several regions in the body (from the head to the tail) indicated that the NO was generated mostly in the upper abdomen near the liver. These observations were confirmed by ex vivo EPR measurements on isolated organs where higher NO levels were detected in vivo in the liver and kidney. The spectroscopic results, combined with the pharmacokinetic data, support the model that NO detected in LPS-treated mice was produced mainly in the liver, and that it did not reflect NO-adduct complex accumulated in the liver via the blood circulation.

Gargas ML, Andersen ME, Teo SKO, Batra R, Fennell TR, Kedderis GL. **A physiologically based dosimetry description of acrylonitrile and cyanoethylene oxide in the rat.** Toxicol Appl Pharmacol 1995;134(2):185-94.
A physiological based dosimetric model for describing the metabolic disposition of acrylonitrile (107131) (ACN) and 2-cyanoethylene-oxide (4538516) (CEO) in the rat was developed. The metabolism and tissue distribution of both compounds were simulated as an eight compartment model consisting of venous and arterial blood, lung, brain, fat, rapidly and slowly perfused tissue, and liver compartments. The model for ACN also incorporated a gastrointestinal tract compartment. The values of the various pharmacokinetic and biochemical parameters were taken from published in-vitro and in-vivo studies and the results of a pharmacokinetic experiment in which F344-rats were injected intravenously with 0 to 84mg/kg ACN or 0 to 5.3mg/kg CEO. The model was used to predict the uptake and disposition of ACN, production and disposition of CEO, urinary excretion of ACN metabolites derived from the epoxidation of ACN to CEO and direct conjugation of ACN with glutathione (GSH), and adduction of hemoglobin (Hb) by ACN and CEO. The predictions were compared with the results obtained in experiments in which male F344-rats were administered 0 to 28mg/kg ACN orally. The rates of ACN epoxidation and GSH conjugation based on the limiting rate for hepatic clearance of ACN and CEO were 1.64 and 0.48 liters per hour (l/hr), respectively. The overall rate of CEO metabolism was calculated to be 11.90l/hr. These values yielded first pass hepatic extraction ratios of 0.61 for ACN and 0.90 for CEO. This result indicated that most of the CEO formed in the liver as a result of ACN epoxidation would be metabolically cleared and not be available to be circulated to extrahepatic target organs. The predicted uptake and disposition of ACN, production and disposition of CEO, urinary elimination of ACN metabolites, and extent of binding of ACN and CEO to Hb agreed well with the experimental results. For example, the extent of binding of 0 to 28mg/kg ACN to Hb was predicted and found experimentally to increase with dose. The maximum binding following treatment with 28mg/kg ACN amounted to 6% of the dose.

Ghoneum M, Vojdani A, Banionis A, Oppenheimer S, Lagos N, Gill G. **The effects of carcinogenic methylcholanthrene on carbohydrate residues of NK cells.** Toxicol Ind Health 1997;13(6):727-41.
The present study examines the effect of methylcholanthrene (MCA), a a carcinogenic polycyclic hydrocarbon, on the carbohydrate receptor determinants (RD) on natural killer (NK) cell surface using the bead-coupled lectin assay. Murine NK cells exhibited different degrees of preferential binding to the specific lectins tested. Of the ten lectins tested, five exhibited a positive binding affinity while the remaining five exhibited no or insignificant binding. NK cells bind to beads derivatized with mannose specific lectins: Concanavalin A (Con A), Lens culinaris, and Pisum sativum. NK cells also bind to other

lectin beads such as Triticum vulgaris (GalNac) and Vicia villosa (D-GlcNAc). All these lectin beads exhibited greater than 90% adhesion. The underivatized control beads exhibited no NK binding. The NK cells that were exposed to MCA for 2 h demonstrated a significant decrease in lectin bead-cell coupling in a dose dependent manner. MCA (10 micrograms/mL) caused a 17.8%, 40% and 4.7% decrease in binding affinity when introduced to the mannose specific lectins; Con A, L. culinaris and P. sativum beads, respectively. The binding of T. vulgaris and V. villosa to NK cells was inhibited (23.4% and 28%) by MCA treatment. An increase in the dose to 20 micrograms/mL resulted in a greater inhibition in binding affinity towards lectin beads. Con A, 35.3%, L. culinaris, 62.6%, P. sativum, 30.9%, T. vulgaris, 44.2% and V. villosa, 46.2%. The effect of MCA activation and cytotoxic response. Hydrolysis of PI metabolites (PIP and PIP2) cause generation of secondary messenger: inositol-1,4,5-triphosphate and diacylglycerol, both of which elicit an immune response through their products (Ca2+ and PKC) respectively. Identification of the relationship between receptor level, induction of second messenger and cytotoxic activity may resolve the molecular basis of suppression of NK cytotoxicity by MCA and other PAH compounds.

Gieschke R, Reigner BG, Steimer JL. **Exploring clinical study design by computer simulation based on pharmacokinetic/pharmacodynamic modeling**. Int J Clin Pharmacol Ther 1997;35(10):469-74.
CBAC COPYRIGHT: CHEM ABS Computer simulations have been successfully applied in various industries (e.g. automobile, aerospace) to make product development more efficient. Just recently, it was suggested to use simulations in support of clin. drug development for predicting clin. outcomes of planned trials. The methodol. basis for this approach is provided by pharmacokinetic and pharmacodynamic math. models together with Monte Carlo techniques. In the present paper, the basic notions of clin. trial simulation are introduced and illustrated with the example of an oral anticancer drug. It is shown that computer simulation helps to evaluate consequences of design features on safety and efficacy assessment of the drug which are not easily obtained otherwise. An overview of existing simulation resources with respect to training and software is provided. This article is reviewed by 22 refs.

Hissink AM, Van Ommen B, Kruse J, Van Bladeren PJ. **A physiologically based pharmacokinetic (PB-PK) model for 1,2-dichlorobenzene linked to two possible parameters of toxicity.** Toxicol Appl Pharmacol 1997;145(2):301-10.
A physiologically based pharmacokinetic (PB-PK) model was developed for 1,2-dichlorobenzene (1,2-DCB) for the rat. This model was adjusted for the human situation, using human in vitro parameters, including a Vmax and Km determined with human microsomes. For comparison, the Vmax and Km values from the rat were scaled allometrically to the human case. The model was used in two ways: (1) Acute hepatotoxicity was related to the amount of reactive metabolites (epoxides) formed in vitro. For rats, the hepatic concentration of epoxide metabolites in vivo after exposure to a toxic dose level (250 mg/kg bw) was predicted using in vitro parameters. For man, the dose level needed to obtain the same toxic liver concentration of reactive metabolites as in rat was predicted, assuming a concentration-effect relationship in the liver. It could be concluded that this concentration is not reached, even after induction of the oxidation step, due to saturation of metabolism and a concomitant accumulation of 1,2-DCB in fat. (2) Hepatotoxicity was related to depletion of glutathione (GSH) in the liver. In the model, the consumption of hepatic GSH by metabolism (based on in vivo and in vitro data) and normal turnover was described. In vivo validation was conducted by comparing the predictions of the model with the

results of a GSH depletion study performed at two dose levels (50 and 250 mg/kg bw). Subsequently, the GSH consumption by 1,2-DCB metabolites was estimated for man using human in vitro metabolic data. GSH turnover in human liver was assumed to be the same as that in rat. It appeared that at a dose level of 250 mg/kg, hepatic GSH was completely depleted after 10 hr for man, whereas for the rat a maximum depletion of 75% was predicted, after 15 hr. The presented model provides a quantitative tool for evaluating human risk for two different toxicity scenarios, namely covalent binding of reactive metabolites and depletion of GSH.

Holland JA, Meyer JW, Schmitt ME, Sauro MD, Johnson DK, Abdul-Karim RW, Patel V, Ziegler LM, Schillinger KJ, Small RF, et al. **Low-density lipoprotein stimulated peroxide production and endocytosis in cultured human endothelial cells: mechanisms of action.** Endothelium 1997;5(3):191-207.
The effects of arachidonic acid metabolism and NADPH oxidase inhibitor on the hydrogen peroxide (H2O2) generation and endocytotic activity of cultured human endothelial cells (EC) exposed to atherogenic low-density lipoprotein (LDL) levels have been investigated. EC were incubated with 240 mg/dl LDL cholesterol and cellular H2O2 production and endocytotic activity measured in the presence and absence of the arachidonic acid metabolism inhibitors, indomethacin, nordihydroguaiaretic acid, and SKF525A, and NADPH oxidase inhibitor, apocynin. All inhibitors, with the exception of indomethacin, markedly reduced high LDL-induced increases in EC H2O2 generation and endocytotic activity. EC exposed to exogenously applied arachidonic acid had cellular functional changes similar to those induced by high LDL concentrations. EC incubated with 1-25 uM arachidonic acid had increased H2O2 production and heightened endocytotic activity. Likewise, EC pre-loaded with [3H]arachidonic acid when exposed to increasing LDL levels (90-330 mg/dl cholesterol) had a dose-dependent rise in cytosolic [3H]arachidonic acid. The phospholipase A2 inhibitors, 4-bromophenacyl bromide and 7,7-dimethyleicosadienoic acid, markedly inhibited H2O2 production in EC exposed to 240 mg/dl LDL cholesterol. These findings suggest that arachidonic acid contributes mechanistically to high LDL-perturbed EC H2O2 generation and heightened endocytosis. Such cellular functional changes add to our understanding of endothelial perturbation, which has been hypothesized to be a major contributing factor in the pathogenesis of atherosclerosis.

Hu X, Singh SV. **Differential catalytic efficiency and enantioselectivity of murine glutathione S-transferase isoenzymes in the glutathione conjugation of carcinogenic anti-diol epoxides of chrysene and benzo(g)chrysene.** Arch Biochem Biophys 1997;345(2):318-24.
BIOSIS COPYRIGHT: BIOL ABS. The kinetics of the conjugation of carcinogenic anti-diol epoxides of chrysene (anti-CDE) and benzo(g)chrysene Lanti-B(g)CDE) with glutathione (GSH) catalyzed by GSH S-transferase (GST) isoenzymes mGSTP1-1, mGSTM1-1, MGSTA3-3, mGSTA4-4, and GST 9.5 of female A/J mouse tissues has been investigated. When GST activity was measured as a function of varying anti-CDE or anti-B(g)-CDE concentrations at a fixed concentration of GSH, each isoenzyme obeyed Michaelis-Menten kinetics. The catalytic efficiencies (kcat/Km) of murine GSTs in the GSH conjugation of anti-CDE were in the order of GST 9.5 > mGSTP1-1 > mGSTM1-1 > mGSTA3-3 > mGSTA4-4. While each GST isoenzyme examined in the present study exhibited preference for the GSH conjugation of (+)-anti-CDE with the (R,S)-diol (SR)-epoxide absolute configuration, which is a far more potent carcinogen than the (-)-anti-CDE ((S,R)-diol (R,S)-epoxide absolute configuration), the

enantioselectivity was relatively more pronounced for mGSTP1-1 compared with other murine GSTs. Anti-B(g)CDE was a relatively poor substrate for each GST isoenzyme examined compared with anti-CDE. The catalytic efficiencies of murine GSTs in the GSH conjugation of anti-B(g)CDE were in the order of GST 9.5 > mGSTP11 > mGSTM1-1 > mGSTA3-3. With the exception of mGSTM1-1, all other murine GSTs exhibited preference for the GSH conjugation of anti-B(g)CDE enantiomer with the (R,S)-diol (SR)-epoxide absolute configuration. In summary, the results of the present study indicate that the murine GSTs significantly differ in their catalytic efficiency and enantioselectivity in the GSH conjugation of both anti-CDE and antiB(g)CDE, and that anti-B(g)CDE is a relatively poor substrate for murine GSTs compared with anti-CDE, which may partially account for the observed relatively higher carcinogenic potency of the former compound.

Ishizaki J, Yokogawa K, Nakashima E, Ichimura F. **Prediction of changes in the clinical pharmacokinetics of basic drugs on the basis of octanol-water partition coefficients**. J Pharm Pharmacol 1997;49(8):762-7.
CBAC COPYRIGHT: CHEM ABS A physiol. based pharmacokinetic model for basic drugs has been established on the basis of octanol-water partition coeffs. of the non-ionized, unbound drugs (Poct). The parameters for the physiol. model in man were estd. from a regression equation obtained for the relationships between the Poct and the tissue-plasma partition coeff., the hepatic intrinsic clearance (CLint,h) and the blood-to-plasma concn. ratio in rabbits. The plasma concns. obsd. after i.v. administration of ten basic drugs (3.2 mg kg-1) to rabbits agreed with the levels predicted using the physiol. model (r=0.710-0.980). In man, the predicted plasma concns. of basic drugs were in good agreement with reported values (r=0.729-0.973), except for diazepam and pentazocine. Variations in plasma and brain-concn. profiles of clomipramine and nitrazepam in various disease states were simulated using the model. The authors assumed that the changes in unbound fraction of drug in serum (fp), CLint,h and the hepatic blood flow rate were from 0.25- to 4-fold that of the control and that fat vol. changed by 0.2- to 5-fold. With regard to changes in fp, the authors predicted that the brain-plasma concn. ratio of clomipramine was 1.5- to 25-fold that of the control 24 h after i.v. administration, although the variations in the plasma concn.-time profiles were less marked. Plasma concns. predicted for several basic drugs were in good agreement with reported values and this physiol. model could be useful for predicting drug-disposition kinetics in man.

Ishizaki J, Yokogawa K, Nakashima E, Ichimura F. **Relationships between the hepatic intrinsic clearance or blood cell-plasma partition coefficient in the rabbit and the lipophilicity of basic drugs**. J Pharm Pharmacol 1997;49(8):768-72.
CBAC COPYRIGHT: CHEM ABS The relationships between drug lipophilicity and hepatic intrinsic clearance (CLint,h) or red blood cell-plasma partition coeffs. (D) have been elucidated for ten highly lipophilic basic drugs with apparent octanol-water partition coeffs. at pH 7.4 (Papp,oct) of 150 or above. The true octanol-water partition coeffs. of the non-ionized drugs (Poct) were used to det. CLint,h and D for the unbound drugs (CLint,h,f and Df, resp.), and CLint,h,f and Df for the non-ionized and unbound drugs (CLint,h,fu and Dfu, resp.). The total clearance values were detd. at steady state by infusion studies of individual drugs in rabbits. There was better correlation between log Poct and log CLint,h,fu (r=0.974) than between log Poct and log CLint,h,f (r=0.864). The D values were calcd. from the blood-plasma concn. ratio. There was a better correlation between log Poct and log Dfu (r=0.944) than between

log Poct and log Df (r=0.612). The regression equations obtained were CLint,h,fu = 0.0875 .times. Poct1.338 and Dfu=0.0108 .times. Poct0.970, resp. These results show that the CLint,h and D of highly lipophilic basic drugs can be predicted from Poct by taking fu into consideration. By applying these parameters to a physiol. based pharmacokinetic model it might be possible to predict the pharmacokinetics of unknown basic drugs.

Klepper O, Bedaux J. **Nonlinear parameter estimation for toxicological threshold models.** Ecol Model 1997;102(2-3):315-24.
BIOSIS COPYRIGHT: BIOL ABS. A number of toxicological models contain a threshold concentration, below which there is no (or background) response. The simplest of these is the 'hockey stick' model, which contains three parameters (threshold, background response and slope), but a variety of more complex and physiologically realistic models containing thresholds have been developed. The threshold makes these models nonlinear in the parameters. Using a number of actual data sets, the paper shows that this nonlinearity may be quite strong, even leading to a confidence interval with multiple disjunct 'compartments'. In this case, the traditional approaches of estimating confidence intervals by ignoring nonlinearity (linearization) or by transformation give unreliable estimates. As an alternative, a robust estimation method is shown to be able to deal with this kind of nonlinearities because it does not rely on an assumed shape of the confidence interval, but produces a sample of points that characterizes this (possibly irregularly shaped) set.

Kubitz R, Schreiber R, Vom Dahl S, Haussinger D. **Ethanol induces vesicular alkalinization in rat hepatocytes**. Cell Physiol Biochem 1997;7(5):289-97.
CBAC COPYRIGHT: CHEM ABS In rat hepatocytes loaded with fluorescein isothiocyanate (FITC)-dextran, EtOH (100 mM) led to a biphasic increase of the pH of endocytotic vesicles (control pHves = 6.11) starting with a peak (DELTApHves = +0.15) followed by a sustained plateau (DELTApHvs = +0.09). The alkalinizing effect of EtOH was fully reversible and was half-maximal at EtOH concns. <5 mM. The effects of EtOH were mimicked by low concns. (0.1 mM) of acetaldehyde, but not by acetate or a lactate-induced shift of the NADH/ NAD+ system to a more reduced state. Inhibition of alc. dehydrogenase by 4-methylpyrazole (2 mM) abolished the effects of EtOH on pHves, but not those of acetaldehyde. The EtOH-induced pHves changes were largely abolished in the presence of colchicine, the chloride channel blocker 4,4'-diiso-thiocyanatostilbene-2,2'-disulfonic acid, pertussis and cholera toxin and the tyrosine kinase inhibitors erbstatin analog and genistein. The results suggest that EtOH via acetaldehyde activates a signal transduction mechanism which triggers an alkalinization of pHves. This may contribute to the known effects of EtOH on the endocytotic pathway.

Lennernaes H. **Human jejunal effective permeability and its correlation with preclinical drug absorption models**. J Pharm Pharmacol 1997;49(7):627-38.
CBAC COPYRIGHT: CHEM ABS A review with _100 refs. This review focuses on intestinal permeability measurements in humans and various aspects of in-vivo transport mechanisms. In addn., comparisons of human data with preclin. models and the blood-brain barrier is discussed. The regional human jejunal perfusion technique has been validated by several crucial points. One of the most important findings is that there is a good correlation between the measured human effective permeability values and the extent of absorption of drugs in humans detd. by pharmacokinetic studies. We have also

shown that it is possible to det. the effective permeability (Peff) for carrier-mediated transported compds., and to classify them according to the proposed Biopharmaceutical Classification System (BCS). Furthermore, it is possible to predict human in-vivo permeability using preclin. permeability models, such as in-situ perfusion of rat jejunum, the Caco-2 model and excised intestinal segments in the Ussing chamber. The permeability of passively transported compds. can be predicted with a particularly high degree of accuracy. However, special care must be taken for drugs with a carrier-mediated transport mechanism, and a scaling factor has to be used. It is also suggested that it is possible to roughly est. the permeability of the blood-brain barrier using measurements of intestinal permeability, even if the quant. role of efflux of P-glycoprotein(s) in-vivo still remains to be clarified. Finally, the data obtained in-vivo in humans emphasize the need for more clin. studies investigating the effect of physiol. in-vivo factors and mol. mechanisms influencing the transport of drugs across the intestinal and as well as other membrane barriers. It is also important to study the effect of anti-transport mechanisms, such as efflux by P-glycoprotein(s), and gut wall metab., for example CYP 3A4, on the bioavailability.

Levin RJ. **Actions of spermicidal and virucidal agents on electrogenic ion transfer across human vaginal epithelium in vitro.** Pharmacol Toxicol 1997;81(5):219-25.

Li AP. **Primary hepatocyte cultures as an in vitro experimental model for the evaluation of pharmacokinetic drug-drug interactions.** Adv Pharmacol 1997;43:103-30.

Li AP, Jurima-Romet M. **Applications of primary human hepatocytes in the evaluation of pharmacokinetic drug-drug interactions: evaluation of model drugs terfenadine and rifampin.** Cell Biol Toxicol 1997;13(4-5):365-74.
The utility of primary human hepatocytes in the evaluation of drug-drug interactions is being investigated in our laboratories. Our initial approach was to investigate whether drug-drug interactions observed in humans in vivo could be reproduced in vitro using human hepatocytes. Two model drugs were studied: terfenadine and rifampin, representing compounds subjected to drug-drug interactions via inhibitory and induction mechanisms, respectively. Terfenadine was found to be metabolized by human hepatocytes to C-oxidation and N-dealkylation products as observed in humans in vivo. Metabolism by human hepatocytes was found to be inhibited by drugs which are known to be inhibitory in vivo. Ki values for the various inhibitors were derived from the in vitro metabolism data, resulting in the following ranking of inhibitory potency: For the inhibition of C-oxidation, ketoconazole > itraconazole > cyclosporin approximately troleandomycin > erythromycin > naringenin. For the inhibition of N-dealkylation, itraconazole > or = ketoconazole > cyclosporin > or = naringenin > or = erythromycin > or = troleandomycin. Rifampin induction of CYP3A, a known effect of rifampin in vivo, was also reproduced in primary human hepatocytes. Induction of CYP3A4, measured as testosterone 6 beta-hydroxylation, was found to be dose-dependent, treatment duration-dependent, and reversible. The induction effect of rifampin was observed in hepatocytes isolated from all 7 human donors studied, with ages ranging from 1.7 to 78 years. To demonstrate that the rifampin-induction of testosterone 6 beta-hydroxylation could be generalized to other CYP3A4 substrates, we evaluated the metabolism of another known substrate of CYP3A4, lidocaine. Dose-dependent induction of lidocaine metabolism by rifampin is observed. Our results suggest that primary human hepatocytes may be a useful experimental system for preclinical evaluation of drug-drug interaction potential during drug development, and as a

tool to evaluate the mechanism of clinically observed drug-drug interactions.

Li S, Marquardt RR, Frohlich AA, Vitti TG, Crow G. **Pharmacokinetics of ochratoxin A and its metabolites in rats.** Toxicol Appl Pharmacol 1997;145(1):82-90.
BIOSIS COPYRIGHT: BIOL ABS. Ochratoxin A (OA) is a mycotoxin that is produced on moist grain. It is commonly found in the blood of swine in western Canada and is a potent nephrotoxic, carcinogen, and immunosuppressive agent. The pharmacokinetic characteristics of six analogs of OA including OA, OB (OA without chloride), OC (OA ethyl ester), and some metabolites, such as Oalpha (OA without phenylalanine), OA-OH (hydroxylated OA), and a newly discovered form of OA, OP-OA (lactone opened ring of OA), were investigated in rats after a single intravenous administration of the compounds. All of the ochratoxin analogs were distributed following a two compartment open model. The elimination half-lives of OA, OP-OA, Oalpha, OA-OH, OB, and OC were 103:16, 50.5-2.8, 9.6:2.3, 6:0.9, 4.2:1.2, and 0.6:0.2 hr, respectively. Total body clearance of OA, OP-OA, Oa, OA-OH, and OB via the bile, urine, and metabolic routes were 3.1, 3.6, 40, 65, and 43 ml/hr kg, respectively. OA, OB, and Oa were mainly cleared in the urine (:48%), OA-OH in the bile (41%), and OP-OA as metabolites (43%). Metabolism accounted for 43, 44, 33, and 29% of the total clearance of OA, Oalpha, OA-OH, and OB, respectively. It is concluded that OA has a long half-life and is very slowly cleared from the body and that its metabolites are cleared at a much faster rate with much shorter half-lives. Procedures should be devised to enhance the conversion in the body of OA to Oalpha, OA-OH, or other metabolites as this would shorten its half-life and therefore its toxicity.

Lim CK, Yuan ZX, Jones RM, White IN, Smith LL. **Identification and mechanism of formation of potentially genotoxic metabolites of tamoxifen: study by LC-MS/MS.** J Pharm Biomed Anal 1997;15 (9-10):1335-42.
On-line high-performance liquid chromatography-electrospray ionization mass spectrometry (HPLC-ESI MS) and tandem mass spectrometry (MS/MS) have been applied to the study of tamoxifen metabolism in liver microsomes and to the identification of potentially genotoxic metabolites. The results showed that the hydroxylated derivatives, including 4-hydroxytamoxifen and alpha-hydroxytamoxifen are detoxication metabolites, while arene oxides, their free radical precursors or metabolic intermediates, are the most probable species involved in DNA-adduct formation.

Lin FT, Krueger KM, Kendall HE, Daaka Y, Fredericks ZL, Pitcher JA, Lefkowitz RJ. **Clathrin-mediated endocytosis of the beta-adrenergic receptor is regulated by phosphorylation/ dephosphorylation of beta-arrestin1.** J Biol Chem 1997;272(49):31051-7.
beta-Arrestins serve a dual regulatory role in the life cycle of G protein-coupled receptors such as the beta2-adrenergic receptor. First, they mediate rapid desensitization by binding to G protein-coupled receptor kinase-phosphorylated receptors. Second, they target the receptors for internalization into endosomal vesicles, wherein receptor dephosphorylation and resensitization occur. Here we report that phosphorylation of a carboxyl-terminal serine (Ser-412) in beta-arrestin1 regulates its endocytotic but not its desensitization function. Cytoplasmic beta-arrestin1 is constitutively phosphorylated and is recruited to the plasma membrane by agonist stimulation of the receptors. At the plasma membrane, beta-arrestin1 is rapidly dephosphorylated, a process that is required for its clathrin binding and receptor endocytosis but not for its receptor binding and desensitization. Once internalized, beta-arrestin1 is

rephosphorylated. Thus, as with the classical endocytic adaptor protein complex AP2, beta-arrestin1 functions as a clathrin adaptor in receptor endocytosis which is regulated by dephosphorylation at the plasma membrane.

Liu Y, Schubert D. **Cytotoxic amyloid peptides inhibit cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction by enhancing MTT formazan exocytosis.** J Neurochem 1997;69(6):2285-93.
Amyloid beta peptide (A beta) neurotoxicity is believed to play a central role in the pathogenesis of Alzheimer's disease. An early indicator of A beta toxicity is the inhibition of cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction to MTT formazan, a widely used assay for measuring cell viability. In this report we show that A beta and other cytotoxic amyloid peptides such as human amylin dramatically enhance MTT formazan exocytosis, resulting in the inhibition of cellular MTT reduction. Only the amyloid peptides that are known to be cytotoxic enhanced MTT formazan exocytosis. Basal MTT formazan exocytosis and amyloid peptide-enhanced MTT formazan exocytosis are blocked by several drugs with diverse known effects. These and other data suggest that MTT formazan exocytosis is a multistep process and that cytotoxic amyloid peptides enhance MTT formazan exocytosis through an intracellular signal transduction pathway.

Luo ZD, Berman HA. **The influence of Pb2+ on expression of acetylcholinesterase and the acetylcholine receptor.** Toxicol Appl Pharmacol 1997;145(2):237-45.
This paper examines the influence of inorganic lead (Pb2+) on the presence of acetylcholinesterase (AchE) molecular forms and the acetylcholine receptor (AchR) in two types of excitable tissue, primary cultures of skeletal muscle and neural retina from embryonic chick. Treatment of skeletal muscle with Pb2+ is observed to cause reductions in the 5/7S and 19S but not the 11.4S molecular forms of AchE. The reductions are dose-dependent, requiring submicromolar concentrations, slow in onset, requiring incubation times greater than 24 hr, and tissue specific, being pronounced in skeletal muscle but absent from neural retina. Significantly, the reductions in AchE occur without corresponding reductions in amounts of AchR and without reduction in activity of protein kinase C (PKC). These studies illustrate a tissue-specific action of inorganic lead that is not mediated through PKC.

Maurin M, Raoult D. **Intracellular organisms**. Int J Antimicrob Agents 1997;9(1):61-70.
CBAC COPYRIGHT: CHEM ABS A review, with 93 refs. The intracellular location of some microorganisms allow them to resist antibiotics with poor ability to penetrate eukaryotic cell membranes, such as the beta-lactam compds. Pharmacokinetic and pharmacodynamic properties of antibiotics within eukaryotic cells have been defined in the last two decades. Penetration but also subcellular localization of antibiotics within cells, as well as the possibility of their intracellular inactivation have to be considered. In vitro infected cell models have been useful to det. the activity of antibiotics against intracellular pathogens, although extrapolation of in vitro exptl. data to the clin. situation remains hazardous. The pharmacokinetic properties and intracellular activity against a no. of pathogens of the newer fluoroquinolone and macrolide compds. seems promising, but clin. data remain scarce.

McCarthy MJ, Rubin LL, Philpott KL. **Involvement of caspases in sympathetic neuron apoptosis.** J

Cell Sci 1997;110(Pt 18):2165-73.
In order to study the involvement of caspases in neuronal cell death, we have examined the effects of the viral caspase inhibitor p35 and peptide caspase inhibitors on sympathetic neurons isolated from the superior cervical ganglion (SCG). In these neurons, apoptosis can be induced by the withdrawal of nerve growth factor (NGF) and also by the addition of the kinase inhibitor staurosporine. p35 has been shown to be a broad spectrum inhibitor of the caspase family and promotes the survival of SCG neurons withdrawn from NGF. We show that p35 is also protective when apoptosis is induced by staurosporine. In addition, p35 inhibits a number of the morphological features associated with apoptosis, such as nuclear condensation, TUNEL labelling, and externalisation of phosphatidylserine. The tri-peptide caspase inhibitor benzyloxycarbonyl-Val-Ala-Asp (O-methyl)-fluoromethylketone (zVAD-fmk) was effective at inhibiting NGF withdrawal-induced and staurosporine-induced apoptosis of SCG neurons. Two other peptide inhibitors, acetyl-Tyr-Val-Ala-Asp-aldehyde (Ac-YVAD-CHO) and acetyl-Asp-Glu-Ala-Asp-aldehyde (Ac-DEVD-CHO), also inhibited apoptosis induced by both means when microinjected into SCG neurons but peptides derived from the caspase cleavage site in p35 were not protective. We present data to suggest that apoptosis induced by separate death stimuli can result either in the activation of distinct caspases or in differences in the time of activation of the family members.

Pereira PT, Monteiro DE Carvalho M, Arrabaca JD, Amaral-Collaco MT, Roseiro JC. **Alternative respiratory system and formamide hydro-lyase activity as the key components of the cyanide-resistance mechanism in Fusarium oxysporum.** Can J Microbiol 1997;43(10):929-36.
BIOSIS COPYRIGHT: BIOL ABS. A strain of Fusarium oxysporum, isolated from an industrial effluent containing a high cyanide concentration, detoxifies cyanide via formamide hydro-lyase (FHL). The importance of the coexistence of the alternative (cyanide insensitive) respiratory system and the synthesis of FHL was assessed. This enzyme, induced by cyanide, converts this compound to formamide and is partially responsible for the tolerance of F. oxysporum to high cyanide concentration. The FHL induction for cyanide detoxification depends on the alternative respiratory system when the terminal oxidase of the electron transport chain is blocked by cyanide used during the induction process. The respiratory metabolism of a F. oxysporum strain was studied. Whole cells exhibited a cyanide-sensitive respiration but developed a partially cyanide-resistant respiration under certain physiological conditions, namely, in the stationary phase of growth, in the presence of chloramphenicol in the growth medium, or upon aeration in the absence of nutrients (starvation conditions). Cycloheximide prevented the appearance of cyanide-insensitive respiration when the cells were aerated under starvation conditions. This fact suggested some form of induction involving de novo protein synthesis.

Poet TS, Borghoff SJ. **In vitro uptake of methyl tert-butyl ether in male rat kidney: use of a two-compartment model to describe protein interactions.** Toxicol Appl Pharmacol 1997;145(2):340-8.
Methyl tert-butyl ether (MTBE) is a gasoline additive that causes renal tumors in male rats. In the process of measuring chemical specific parameters necessary to develop a quantitative dosimetry model of MTBE in rats, the uptake of MTBE was found to be 5.5 times greater in male than in female F-344 rat kidney homogenate. The objectives of this study were to characterize the factor(s) that influences the high uptake of MTBE into male rat kidney in vitro and to develop a system to evaluate the interaction of MTBE with the male rat-specific protein, alpha 2u-globulin (alpha 2u). The uptake of MTBE in male, but not female, rat kidney homogenate was found to be dependent on protein and chemical

concentrations. When [14C]MTBE was incubated with male rat kidney homogenate, radioactivity coeluted with the total protein fraction on a gel filtration column. An interaction between [14C]MTBE and male rat kidney proteins was not found under conditions of dialysis or anion exchange chromatography. A two-compartment vial equilibration model was used to assess the interaction between MTBE and alpha 2u. Using this system, the dissociation constant for MTBE and alpha 2u was estimated to be 2.15 x 10(-4) M, which is in the range of other chemicals known to bind to alpha 2u and cause alpha 2u-mediated nephropathy. d-Limonene oxide was used to validate this two-compartment vial equilibration system. These findings illustrate a technique useful in estimating the dissociation constant for a volatile chemical and a protein, as well as explain the process that contributes to the uptake of MTBE into male rat kidney homogenate in vitro. A description of the weak interaction between MTBE and alpha 2u will be used to refine a physiologically based pharmacokinetic model to describe the target tissue (kidney) concentrations of MTBE.

Porter DW, Yakushiji H, Nakabeppu Y, Sekiguchi M, Fivash MJ Jr, Kasprzak KS. **Sensitivity of Escherichia coli (MutT) and human (MTH1) 8-oxo-dGTPases to in vitro inhibition by the carcinogenic metals, nickel(II), copper(II), cobalt(II) and cadmium(II).** Carcinogenesis 1997;18 (9):1785-91.
The toxicity of Ni(II), Co(II) and Cu(II) in animals, and that of Cd(II) in cultured cells, has been associated with generation of the promutagenic lesion 8-oxo-7,8-dihydroguanine (8-oxoguanine) in DNA, among other effects. One possible source of this base may be 8-oxo-7,8-dihydro-2'-deoxyguanosine-5'-triphosphate (8-oxo-dGTP), a product of oxidative damage to the nucleotide pool, from which it is incorporated into DNA. To promote such incorporation, the metals would have to inhibit specific cellular 8-oxo-dGTPases that eliminate 8-oxo-dGTP from the nucleotide pool. The present study was designed to test such inhibition in vitro on 8-oxo-dGTPases from two different species, the human MTH1 protein and Escherichia coli MutT protein. In the presence of Mg(II), the natural activator of 8-oxo-dGTPases, all four metals were found to inhibit both enzymes. For MTH1, the IC50 values (+/- SE; n = 3-4) were 17 +/- 2 microM for Cu(II), 30 +/- 8 microM for Cd(II), 376 +/- 71 microM for Co(II) and 801 +/- 97 microM for Ni(II). For MutT, they were 60 +/- 6 microM for Cd(II), 102 +/- 8 microM for Cu(II), 1461 +/- 96 microM for Ni(II) and 8788 +/- 1003 microM for Co(II). Thus, Cu(II) and Cd(II) emerged as much stronger inhibitors than Ni(II) and Co(II), and MTH1 appeared to be generally more sensitive to metal inhibition than MutT. Interestingly, in the absence of Mg(II), the activity of the enzymes could be restored by Co(II) to 73% of that with Mg(II) alone for MutT, and 34% for MTH1, the other metals being much less or non-effective. The difference in sensitivity to metal inhibition between the two enzymes may reflect the differences in the amino acid ligands, especially the cysteine ligand, outside their evolutionarily conserved Mg(II)-binding active sites, which might indicate predominantly non-competitive or uncompetitive mechanism of the inhibition. The overall results suggest that inhibition of 8-oxo-dGTPases may be involved in the mechanisms of induction of the 8-oxoguanine lesion in DNA by the metal ions studied, especially the non-redox-active Cd(II) cation.

Ray WJ, Bain G, Yao M, Gottlieb DI. **CYP26, a novel mammalian cytochrome P450, is induced by retinoic acid and defines a new family**. J Biol Chem 1997;272(30):18702-8.
CBAC COPYRIGHT: CHEM ABS A novel member of the cytochrome P 450 superfamily, CYP26, which represents a new family of cytochrome P 450 enzyme,s has been cloned. CYP26 mRNA is

upregulated during the retinoic acid (RA)-induced neural differentiation of mouse embryonic stem cells in vitro and is transiently expressed by embryonic stem cells undergoing predominantly non-neural differentiation. CYP26 transcript is detectable as early as embryonic day 8.5 in mouse embryos, suggesting a function for the gene in early development. CYP26 expressed in mouse and human liver, as expected for a cytochrome P 450, and is also expressed in regions of the brain and the placenta. Acute administration of 100 mg/kg all-trans-RA increases steady-state levels of transcript in the adult liver, but not in the brain. CYP26 is highly homologous to a Zebrafish gene, CYPRA1, which has been proposed to participate in the degrdn. of RA, but is minimally homologous to other mammalian cytochrome P 450 proteins. Thus, the authors report the cloning of a member of a novel cytochrome P 450 family that is expressed in mammalian embryos and in brain and is induced by RA in the liver.

Roth A, Kreienkamp H, Meyerhof W, Richter D. **Phosphorylation of four amino acid residues in the carboxyl terminus of the rat somatostatin receptor subtype 3 is crucial for its desensitization and internalization**. J Biol Chem 1997;272(38):23769-74.
CBAC COPYRIGHT: CHEM ABS Agonist-dependent internalization of the rat somatostatin receptor subtype 3 (SSTR3) requires four hydroxyl amino acids (Ser341, Ser346, Ser351, and Thr357) in the receptor C terminus (Roth, A., et al. (1997)). Here we report on the mol. mechanism responsible for the endocytotic process by analyzing the agonist-dependent phosphorylation of wild-type and mutant receptors expressed in human embryonic kidney cells. Wild-type SSTR3 is phosphorylated in response to agonist treatment. Phosphorylation is markedly reduced in a S341A/S346A/S351A triple mutant and is also reduced, but to a lesser extent, in the T357A point mutant. Internalization of the wild-type receptor is preceded by a functional desensitization of the receptor; in contrast, the triple serine mutant does not desensitize after treatment with agonists as assayed by its ability to inhibit forskolin-stimulated adenylate cyclase activity. After internalization via a clathrin-coated vesicle mediated endocytotic pathway, SSTR3 efficiently recycles to the cell surface, suggesting that agonist mediated endocytosis is necessary for the functional resensitization of a phosphorylated and desensitized receptor.

Sauviat MP. **[Effect of neurotoxins on the electrical and mechanical activity of heart muscle]**. Comptes Rend Seances Soc Biol Filiales 1997;191(3):451-71. (Fre)
BIOSIS COPYRIGHT: BIOL ABS. The goal of the present review is to report the of the main neurotoxins known on the electrical and mechanical activity of heart muscle. Toxins which block the sodium channel (tetrodotoxin, saxitoxin) shorten the action potential (AP) duration and decrease the initial depolarizing phase of the AP. Toxins which occupy different sites in the channel and alter the gating mechanisms of the Na channel (aconitine, batrachotoxin, veratridine, sea anemone and scorpion toxins, brevetoxin and ciguatoxin) depolarize, lengthen the AP duration, increase the contraction and cause arrhythmias. Ca channel agonists (atrotoxin, maitotoxin) increase the amplitude of the cardiac plateau. Ca channel antagonists (TCX, co-conotoxin) decrease the magnitude of the plateau and exert a negative inotropic effect. Okadaic acid increases the Ca current leading to an increase in the plateau amplitude and a lengthening in the AP duration and the development of a positive inotropic effect on the contraction. Toxin affecting voltage-dependent K channels on heart muscle and the actual knowledge concerning the effect and the mode of action of palytoxin have also been reviewed. It is concluded that toxins, used as tools to analyze and characterize the structure and the function of ionic channels involved in the development of the electrical activity of excitable cells exhibit numerous effects on cardiac

muscle. Some of these effects might not only be due to a direct action of these substances on membrane channels but they might also be the result of the release of neuromediators from nervous endings surrounding cardiac cells.

Schueuermann G, Segner H, Jung K. **Multivariate mode-of-action analysis of acute toxicity of phenols.** Aquatic Toxicol 1997;38(4):277-96.
BIOSIS COPYRIGHT: BIOL ABS. Acute toxicity of eight phenols towards ten biological test systems is characterized with principal component analysis and partial least-squares regression in terms of underlying modes of action and associated compound properties. The test battery consists of nine different species: three fish, one waterflea, one ciliate, one marine bacterium in two different assays, two fish cell lines and one plant pollen. The compound set contains phenol, five chlorophenols and two nitrophenols, representing polar narcotics and uncouplers of oxidative phosphorylation. Lipophilicity (log Kow) and acidity (pKa) as well as respective bilinear decompositions in quantum chemical parameters are used as molecular descriptors. The results reveal substantial differences in specific sensitivity of the test systems for uncoupling activity. Furthermore, pentachlorophenol (as a classical uncoupler) exerts no significant excess toxicity over polar narcosis with any of the ten endpoints, and 4-nitrophenol apparently acts as uncoupler with most of the non-fish test species including procaryotic cells. The discussion includes alternative hypotheses about the underlying mechanism of uncoupling activity.

Shen L, Shah AM, Dahlback B, Nelsestuen GL. **Enhancing the activity of protein C by mutagenesis to improve the membrane-binding site: studies related to proline-10.** Biochemistry 1997;36 (51):16025-31.
Bovine and human protein C show high homology in the amino acids of their GLA domains (amino-terminal 44 residues), despite the about 10-fold higher membrane affinity of the human protein. A proposed membrane contact site and mechanism suggested that this difference was largely due to the presence of proline at position 10 of bovine protein C versus histidine at position 10 of human protein C [McDonald, J.F., Shah, A.M., Schwalbe, R.A., Kisiel, W., Dahlback, B., and Nelsestuen, G.L. (1997) Biochemistry, 36, 5120-5127]. This study examined the impact of replacing proline-10 in bovine protein C with histidine, and the reverse change in human protein C. In both cases, the protein containing proline-10 showed lower membrane affinity, about 10-fold lower for bovine protein C and 5-fold lower for human protein C. As expected, activated human protein C (hAPC) containing proline at position 10 showed 2.4-3.5-fold lower activity than wild type hAPC, depending on the assay used. Most interesting was that bovine APC containing histidine-10 displayed up to 15-fold higher activity than wild type bAPC. This demonstrated the ability to improve both membrane contact and activity by mutation. This general strategy should be applicable to other vitamin K-dependent proteins, providing opportunities to study function as well as to produce proteins that may find use as promoters and inhibitors of blood coagulation in pathological states.

Sherratt PJ, Pulford DJ, Harrison DJ, Green T, Hayes JD. **Evidence that human class theta glutathione S-transferase T1-1 can catalyse the activation of dichloromethane, a liver and lung carcinogen in the mouse. Comparison of the tissue distribution of GST T1-1 with that of classes Alpha, Mu and Pi GST in human.** Biochem J 1997;326(3):837-46.

BIOSIS COPYRIGHT: BIOL ABS. The cDNA encoding human glutathione S-transferase (GST) T1 has been expressed as two recombinant forms in Escherichia coli that could be purified by affinity chromatography on either IgG-Sepharose or nickel-agarose; one form of the transferase was synthesized from the pALP 1 expression vector as a Staphylococcus aureus protein A fusion, whereas the other form was synthesized from the pET-20b expression vector as a C-terminal polyhistidine-tagged recombinant. The yields of the two purified recombinant proteins from E. coli cultures were approx. 15 mg/l for the protein A fusion and 25 mg/l for the C-terminal polyhistidine-tagged GST T1-1. The purified recombinant proteins were catalytically active, although the protein A fusion was typically only 5-30% as active as the histidine-tagged GST. Both recombinant forms could catalyse the conjugation of glutathione with the model substrates 1,2-epoxy-3-(4'-nitrophenoxy)propane, 4-nitrobenzyl chloride and 4-nitro-phenethyl bromide but were inactive towards 1-chloro-2,4-dinitrobenzene, ethacrynic acid and 1-menaphthyl sulphate. Recombinant human GST T1-1 was found to exhibit glutathione peroxidase activity and could catalyse the reduction of cumene hydroperoxide. In addition, recombinant human GST T1-1 was found to conjugate glutathione with dichloromethane, a pulmonary and hepatic carcinogen in the mouse. Immunoblotting with antibodies raised against different transferase isoenzymes showed that GST T1-1 is expressed in a large number of human organs in a tissue-specific fashion that differs from the pattern of expression of classes Alpha, Mu and Pi GST. Most significantly, GST T1-1 was found in only low levels in human pulmonary soluble extract of cells, suggesting that in man the lung has little capacity to activate the volatile dichloromethane.

Shim JY, Richard AM. **Theoretical evaluation of two plausible routes for bioactivation of S-(1,1-Difluoro-2,2-dihaloethyl)-L-cysteine conjugates: thiirane vs thionoacyl fluoride pathway**. Chem Res Toxicol 1997;10(1):103-10.

Possible mechanisms for the bioactivation of S-(1,1-difluoro-2,2-dihaloethyl)-L-cysteine conjugates were evaluated. The two metabolic pathways, in which the conjugates were converted to thiolates, differed according to the involvement of either a thiirane or thionoacyl fluoride intermediate. The pathway preferences of fluorinated, chlorinated, and brominated 2,2-dihalo-1,1-difluoroethane-1-thiolates (2,2-dihalo-DFETs) were established by assessing the transition state (TS) geometries, reaction potential energy profiles (PEPs), and activation energies of the compounds. Overall, the low energy level geometries were similar to the high level geometries. The thiirane pathway exhibited a planar geometry. The range of TS imaginary vibrational frequencies was greater for the thiirane pathway than for the thionoacyl fluoride pathway. At the lowest level of theory tested, the PEPs of the thionoacyl fluoride pathway, 86 to 89 kilocalories per mole (kcal/mol), were significantly higher than those of the thiirane pathway, 19 to 31kcal/mol. However, at the higher level of theory tested, the PEPs of thionoacyl fluoride pathway were 30 to 50kcal/mol lower than those of the thiirane pathway. When the lower theory level was tested, chlorine was considered a better leaving atom for the thiirane pathway than bromine. In contrast, the activation energies of the atoms were lowest for bromine, higher for chlorine, and highest for fluoride for the thiirane pathway based on the higher theory level. Only the 2,2-difluoro-DFET preferred the thionoacyl fluoride pathway. By employing even higher levels of theory, the energy barrier of the thionoacyl fluoride pathway applied to the 2,2-difluoro-DFET decreased from 62.7 to 34kcal/mol, and the relative energy preference increased from 3.07 to 8.79kcal/mol. A thionoacyl fluoride pathway was predicted for 2,2-difluoro-DFET, whereas a thiirane pathway was predicted for the brominated 2,2-dihalo-DFETs. The authors conclude that computational models, as complements to

experimental findings, are appropriate for assessing the feasibility of metabolic pathways.

Timchalk C, Dryzga MD, Johnson KA, Eddy SL, Freshour NL, Kropscott BE, Nolan RJ. **Comparative pharmacokinetics of (14C)metosulam (N-(2,6-dichloro-3-methylphenyl)-5,7-dimethoxy-1,2,4-triazolo-(1, 5a)-pyrimidine-2-sulfonamide) in rats, mice and dogs**. J Appl Toxicol 1997;17(1):9-21. The pharmacokinetics of carbon-14 (C14) labeled metosulam (139528851) (C14-metosulam) were determined in a comparative study using rats, mice, and dogs. Male Sprague-Dawley-rats, CD1-mice, and Beagle-dogs were gavaged orally with a single dose of 100mg/kg C14-metosulam. Two dogs were given a second oral dose of 100mg/kg C14-metosulam and killed 12 hours (hr) after dosing, while a third dog was given an intravenous dose of 1mg/kg C14-metosulam for the analysis of plasma, urine, and fecal samples 72hr after dosing. Blood, urine, feces, and tissue samples were collected at up to 168hr postdosing for rats and mice, and up to 216hr postdosing for dogs. Urine and plasma samples were analyzed for metabolites using high performance liquid chromatography, and tissue samples were assessed for C14 activity as well as localization of C14 via histoautoradiography. One or two compartment pharmacokinetic models were used to describe the time course of C14-metosulam in the plasma of the rat, mouse, and dog. In all three species, C14-metosulam was absorbed rapidly; mice and dogs absorbed approximately 20% of the isotope, while rats absorbed over 70%. In the dog, the retina showed affinity for the radiotracer as indicated by analysis of C14 activity and histoautoradiography of dog eyes. No evidence of C14 localization was seen in dog kidneys or in the eyes of rats. The radiotracer and its metabolites were excreted in the urine of all three species, and the relative amount of C14-metosulam metabolism was greatest in mice, followed by rats, and dogs. Half lives for rapid initial and slower terminal phases were 20 and 155hr for mice, and 9 and 60hr for rats, while dogs had an elimination half life of 73hr. The authors conclude that C14-metosulam is not completely absorbed in mice and dogs after oral administration, and that dogs do not extensively metabolize the radioisotope compared to rats and mice, which may explain the selective affinity of metosulam to dog retinas.

Troxel CM, Buhler DR, Hendricks JD, Bailey GS. **CYP1A induction by beta-naphthoflavone, aroclor 1254, and 2,3,7,8-tetrachlorodibenzo-p-dioxin and its influence on aflatoxin B1 metabolism and DNA adduction in zebrafish (Danio rerio).** Toxicol Appl Pharmacol 1997;146(1):69-78. BIOSIS COPYRIGHT: BIOL ABS. This study investigated the inductive response of cytochrome P4501A (CYP1A) in the zebrafish (Danio rerio) following exposure to Aroclor 1254, beta-naphthoflavone (betaNF), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and then investigated TCDD modulation of aflatoxin B1 (AFB1) metabolism and hepatic AFB1-DNA adduction. Aroclor 1254 fed at 500 ppm for 1 to 9 days or intraperitoneal (ip) injection of 75-200 mg Aroclor 1254/kg body weight faded to induce CYP1A protein or associated 7-ethoxyresorufin-O-deethylase (EROD) activity. By contrast, dietary betaNF at 500 ppm for 3 or 7 days induced CYP1A protein and EROD activity approximately threefold above controls. A single ip injection of 150 mg/kg betaNF showed maximal induction of CYP1A protein and EROD activity near 24 hr, both of which decreased to control levels during the next 6 days. Single ip administration of 25, 50, 100, or 150 mg betaNF/kg body weight provided dose-responsive increases in CYP1A and EROD activity. Dietary exposure to 0.75 ppm TCDD for 3 days also significantly induced CYP1A and EROD. The effect of TCDD on the metabolism of (3H) AFB1 in zebrafish was then investigated. The major (3H)AFB1 metabolites excreted in water over 24 hr in the control group were aflatoxicol, aflatoxicol-glucuronide, and parent AFB1. By contrast, the

predominant metabolites in the TCDD-pretreated group were aflatoxicol-M1-glucuronide, aflatoxicol, aflatoxin M1 plus aflatoxicol-M1 (unresolved), aflatoxicol-glucuronide, and parent AFB1. Surprisingly, hepatic AFB1-DNA adduction was approximately fourfold higher in the TCDD treated group than in controls. This significant difference could not be explained by increased capacity for bioactivation of AFB, as measured by an in vitro AFB1-exo-8,9-epoxide trapping assay. However, it was demonstrated that both control and induced zebrafish have high capacity to bioactivate aflatoxin M1 to a reactive intermediate, such that secondary bioactivation of this genotoxic intermediate may be responsible for the increased DNA binding.

Van Der Graaf PH, Danhof M. **Analysis of drug-receptor interactions in vivo. A new approach in pharmacokinetic-pharmacodynamic modeling**. Int J Clin Pharmacol Ther 1997;35(10):442-6.
CBAC COPYRIGHT: CHEM ABS Anal. of pharmacodynamic data using the empirical Hill equation only provides limited insights in the underlying factors that det. the shape and location of a concn.-effect curve, such as agonist affinity and efficacy. We have developed a method which allows for the estn. of agonist affinity and efficacy in vivo and yields more insight in the factors that det. pharmacodynamic variability. The method is based on the operational model of agonism, which describes agonist concn.-effect curves in terms of the max. system effect (Em), the slope of the transducer function (n), the agonist dissocn. equil. const. (KA) and an efficacy parameter (.tau.). We applied the model to obtain ests. of apparent affinity and efficacy of a series of N6-cyclopentyladenosine (CPA) analogs for adenosine A, receptor-mediated in vivo effects on heart rate in rat. In all cases, the model converged and ests. of apparent affinity (pKA) and efficacy (tau) were obtained which were highly consistent with results from in vitro radioligand-binding studies. In conclusion, we have shown that the operational model of agonism can provide meaningful measures of agonist affinity and efficacy in vivo. The model may serve as a practical guide for future development of partial adenosine A1 receptor agonists and help to elucidate the mechanisms underlying adenosine A1 receptor-mediated responses in vivo.

Wang X, Santostefano MJ, Evans MV, Richardson VM, Diliberto JJ, Birnbaum LS. **Determination of parameters responsible for pharmacokinetic behavior of TCDD in female Sprague-Dawley rats.** Toxicol Appl Pharmacol 1997;147(1):151-68.
BIOSIS COPYRIGHT: BIOL ABS. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is the most toxic member of a class of planar and halogenated chemicals. Improvements in exposure assessment of TCDD require scientific information on the distribution of TCDD in target tissues and cellular responses induced by TCDD. Since 1980, several physiologically based pharmacokinetic (PBPK) models for TCDD and related compounds have been reported. Some of these models incorporated the induction of a hepatic binding protein in response to interactions of TCDD, the Ah receptor, and DNA binding sites and described the TCDD disposition in a biological system for certain data sets. Due to the limitations of the available experimental data, different values for the same physical parameters of these models were obtained from the different studies. The inconsistencies of the parameter values limit the application of PBPK models to risk assessment. Therefore, further refinement of previous models is necessary. This paper develops an improved PBPK model to describe TCDD disposition in eight target tissues. The interaction of TCDD with the Ah receptor and with hepatic inducible CYP1A2 were also incorporated into the model. This model accurately described the time course distribution of TCDD following a single oral dose of 10 mug/kg, as well as the TCDD concentration on Day 3 after six different doses, 0.01, 0.1,

0.3, 1, 10, and 30 mug TCDD/kg, in target tissues. This study extends previous TCDD models by illustrating the validity and the limitation of the model and providing further confirmation of the potential PBPK model for us in optimal experimental design and extrapolation across doses and routes of exposure. In addition, this study demonstrated some critical issues in PBPK modeling.

Wen PH, Blumenthal KM. **Structure and function of Cerebratulus lacteus neurotoxin B-IV: tryptophan-30 is critical for function while lysines-18, -19, -29, and -33 are not required.** Biochemistry 1997;36(43):13435-40.
The Cerebratulus lacteus B-toxins are a family of polypeptide neurotoxins known to bind to crustacean voltage-sensitive sodium channels. We have previously shown that in the most abundant homolog, toxin B-IV, Arg-17 in the N-terminal helix and a positive charge at position 25 in the loop region are essential for function. In this report, we target a tryptophan residue at position 30, as well as lysine residues found in both the N-terminal helix and loop regions by polymerase chain reaction mutagenesis, to determine their contributions to toxin activity. Substitution of Trp-30 with a serine causes a more than 40-fold reduction in specific toxicity, whereas replacement by tyrosine and phenylalanine is well tolerated. The secondary structures of both these muteins are identical to that of the wild-type toxin as determined by circular dichroism spectroscopy. Thermal denaturation experiments also show that their conformational stabilities are intact. These results demonstrate that an aromatic residue at this position is required for toxin function. Charge neutralizing substitutions of Lys-18 and Lys-19 located in the N-terminal helix have very little effect on toxicity, suggesting the nonessentiality of these residues. Similar results are also obtained for the charge neutralizing muteins for Lys-29 and Lys-33 in the loop region. Interestingly, reduction experiments demonstrate that both K29N and W30S are more sensitive to reducing agent than wild-type B-IV, raising the possibility that the loop sequence may modulate toxin stability.

Wu G. **Use of a five-compartment closed model to describe the effects of ethanol inhalation on the transport and elimination of injected pyruvate in the rat.** Alcohol Alcohol 1997;32(5):555-61.
BIOSIS COPYRIGHT: BIOL ABS. A five-compartment closed model was established using the system of differential equations. The completely analytical solution of a five-compartment closed model was found using the Laplace transform. 2-(14C)Pyruvate kinetics were studied in rats without and with inhalation of vaporized ethanol, and were modelled by the five-compartment closed model, i.e. injected site, blood, eliminated 14CO2 in air, eliminated 14C in urine and faeces. The kinetic parameters were estimated using the analytical solution of the five-compartment closed model to fit eliminated 14CO2, and 14C in urine and faeces simultaneously. The compartmental analysis showed that the inhalation of vaporized ethanol can increase 2-(14C)pyruvate trans-membrane, trans-tissue processes and oxidation rate. The model developed is useful for general pharmacokinetic and toxicokinetic analysis as well as for studies on ethanol.

PULMONARY TOXICITY

Cotgreave IA. **Absorption and metabolic fate of ozone. The molecular basis of ozone-induced toxicity**. Scand J Work Environ Health 1996;22(Suppl 3):14-26.
The purpose of this review was to allow a better appreciation of the complexity of molecular mechanisms by which ozone (10028156) harms the pulmonary system and to understand how much is

yet to be learned. The first section of the review discussed the interplay between ozone and reactive oxygen metabolites such as superoxide, hydrogen-peroxide, and the hydroxy radical along with other free radicals. The second part examined the chemical reactivity of ozone and progeny reactive species and demonstrated how such species form the basis for the reaction of ozone with biological molecules, particularly with lipids, proteins, and nucleic acids. Thirdly, the review discussed the potential of such changes to important biological molecules interfering with biochemical processes in cells, resulting in cell phenotype, cytotoxicity and genotoxicity. The chemical reactivity of ozone and its rapid degradation to reactive oxygen metabolites facilitate a reaction with most classes of biological macromolecules. This reactivity likely restricts the existence of ozone to the lumen of the lung after the gas is inhaled. Reactions with polyunsaturated fatty acids in biological membranes, the induction of lipid peroxidation, and the release of biologically active metabolites may be important in both the acute cytolytic toxicity of the gas and the development of chronic toxicity. The interaction of ozone with other cellular macromolecules is less well understood.

Hornberg C, Maciuleviciute L, Seemayer NH. **Comparative analysis of cyto- and genotoxic effects of airborne particulates on human and rodent respiratory cells in vitro.** Toxicol In Vitro 1997;11 (5):711-5.
BIOSIS COPYRIGHT: BIOL ABS. In our highly industrialized world air pollution has become an important topic. Beside gaseous pollutants airborne particulates are of great medical concern, containing several hundred mostly organic substances. They are incriminated to cause an excess mortality. Airborne particulates were collected in the heavily industrialized Ruhr region utilizing a high volume sampler HVS 150 (Strohlein Instruments) equipped with glass fibre filters. Chemical substances were extracted with dichloromethane and quantitatively transferred to dimethyl sulfoxide for tissue culture experiments. Cytotoxicity of extracts was determined by reduction of 'plating efficiency' of human cell line A-549 (pneumocyte type II). The induction of 'sister chromatid exchanges' was used as a sensitive bioassay for detection of genotoxic activity of airborne particulates. As target cells we utilized tracheal epithelial cells of the Syrian golden hamster and the rat, human bronchial epithelial cells of line BEAS-2B and human lymphocytes. Quantities of substances equivalent to airborne particulates from 4 and more ml air exerted cytotoxic effects, while quantities of substances from 0.5 ml of air were markedly genotoxic.

Hornberg C, Maciuleviciute L, Seemayer NH. **[Comparative study on cytotoxic and mutagenic effect of airborne dust particles on humand and animal lung and tracheo-bronchial cells]**. Atemwegs Lungenkr 1997;23(7):372-4. (Ger)
BIOSIS COPYRIGHT: BIOL ABS. RRM RESEARCH ARTICLE HUMAN HAMSTER RAT PATIENT RESPIRATORY SYSTEM AIRBORNE DUST PARTICLES TRACHEOBRONCHIAL CELLS EPITHELIAL CELLS LUNG SISTER CHROMATID EXCHANGE POLLUTION MUTAGENESIS CYTOTOXICITY RESPIRATORY SYSTEM.

Krejcie TC, Avram MJ, Gentry WB, Niemann CU, Janowski MP, Henthorn TK. **A recirculatory model of the pulmonary uptake and pharmacokinetics of lidocaine based on analysis of arterial and mixed venous data from dogs.** J Pharmacokinet Biopharm 1997;25(2):169-90.
Pulmonary uptake of basic amine xenobiotics such as lidocaine may influence the onset of drug effect and ameliorate toxicity. To date, pharmacokinetic analysis of pulmonary drug uptake has been only

semiquantitative and ill-suited for relating pharmacodynamics to pharmacokinetics or for estimating the time course of the fraction of drug dose residing in the lung during a single pass. We have developed recirculatory models in an experiment in which lidocaine was injected into the right atrium simultaneously with markers of intravascular space (indocyanine green) and total body water (antipyrine); this was followed by rapid arterial and mixed venous blood sampling. Such models are interpretable physiologically and are capable of characterizing the kinetics of the pulmonary uptake of lidocaine in addition to peripheral tissue distribution and elimination. The apparent pulmonary tissue volume of lidocaine (39 ml/kg) was nearly ninefold greater than that of antipyrine (4.5 ml/kg). The recirculatory model characterized both arterial and mixed venous data, but the latter data were not essential for estimating lidocaine's pulmonary disposition either before or after recirculation of drug was evident.

Patton GW, Paciga JE, Shelley SA. **NR8383 alveolar macrophage toxic growth arrest by hydrogen peroxide is associated with induction of growth-arrest and DNA damage-inducible genes GADD45 and GADD153.** Toxicol Appl Pharmacol 1997;147(1):126-34.
BIOSIS COPYRIGHT: BIOL ABS. Breathing air exposes humans and other mammals to various toxic agents including oxidative contaminants associated with fine particles of less than 2.5 muM which may be deposited in the deep lung and have been implicated in the increased morbidity and mortality correlated with air pollution. Oxidative damage from inhaled particles may include damage to DNA, thereby adversely affecting the immunosurveillance provided by alveolar macrophages. Using the rat alveolar macrophage cell line NR8383, we demonstrated that cell proliferation was inhibited by exogenous hydrogen peroxide, an oxidant naturally produced in cellular respiration and phagocytosis. Mercaptosuccinate, a specific inhibitor of the antioxidant enzyme glutathione peroxidase, also inhibited cell growth. Genes known to be coordinatively regulated in response to growth arrest and DNA damage, GADD45 and GADD153, were induced compared to the housekeeping gene beta-ACTIN by equitoxic doses of hydrogen peroxide and mercaptosuccinate Hydrogen peroxide treatment of cells in which glutathione peroxidase was inhibited by mercaptosuccinate resulted in even greater induction of both GADD genes. This approach using the NR8383 alveolar macrophage cell line provides a model for studying genotoxicity at the mechanistic level at which stress-responsive genes involved in growth arrest and DNA-damage response are modulated.

QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIPS

Adenot M, Benezech V, Bompart J, Bonnet P, Chapat J, Grassy G. **Interest of cluster significance analysis in structure-affinity relationships for non-xanthine heterocyclic antagonists of adenosine**. Eur J Med Chem 1997;32(6):493-504.
CBAC COPYRIGHT: CHEM ABS To define some predictive rules for the discrimination of adenosine antagonists by their A1-receptor affinity, the authors performed a systematic QSAR anal. As no significant descriptors of affinity were found, the authors then proposed to introduce a calcd. enthalpy or entropy change for the interaction as a first approxn. of the affinity descriptors. Since the structural details of the common receptor binding site remain to be detd., an indirect strategy was utilized involving the simulation of amino acid residues that are thought to interact with the ligand. Estg. enthalpic and entropic components by means of a semi-empirical quantum mech. AM1 force calcn., the

authors found a significant clustering of enthalpy change values. This method provides a good descriptor of interaction and also a simple tool for testing hypotheses on the nature of putative binding sites.

Benigni R, Giuliani A. **QSAR approaches in mutagenicity and carcinogenicity estimation**. In: Van De Waterbeemd H; Testa B; Folkers G, Editors. Computer-Assisted Lead Finding and Optimization: Current Tools for Medicinal Chemistry. New York: Wiley-VCH; 1997. P. 293-312.
CBAC COPYRIGHT: CHEM ABS A review and discussion with 59 refs. Although QSAR is primarily a modern technique pioneered in the drug industry, the concept of the relation between chem. structure and biol. activity was established in the nineteenth century in the area of toxicol. Moreover, after the modern formulation of these ideas in the 1960's in the field of medicinal chem., it soon became apparent that the QSAR models could be used to formalize and investigate any type of biol. activity, including toxicity. The frequency and the systematicness with which the QSAR approaches are used in medicinal chem. are unparalled in toxicol. There are also great differences in the use of QSAR in the various areas of toxicol. This work presents the QSAR studies produced for the mutagenicity and carcinogenicity endpoint. As in classical QSAR applications to medicinal chem., various studies relative to individual classes of mutagens and carcinogens describe how the potency of the active chems. in each class varies according to the variation of chem. structure/properties. However, in mutagenicity and carcinogenicity QSAR studies, the need for investigating the difference between active and nonactive chems. has a primary importance, since risk assessment is concerned first with this issue, and second with the potency of the active compds. A no. of examples show how fundamentally types of QSAR models may exist for the same chem. class: those that model the conditions for distinguishing between activity classes e.g., actives and inactive, and those that model the conditions for modulating potencies among the actives. Moreover, the needs relative to the practice of risk assessment have motivated attempts to construct general QSAR models (e.g., for predicting chem. carcinogenicity), not tailored to congeneric series of chems., with the ambitious hope that these models would be valid for all kind of chems. The performance of these attempts in validation studies has usually been limited, and the results of the predictions for the individual chems. have shown that the various SAR and QSAR approaches essentially acted as gross class-identifiers: they pointed to the presence or absence of alerting chem. functionalities, but were not able to make gradations within each potentially harmful class. The possible reasons of these limitations, together with future perspectives are discussed.

Bruggemann R, Niederfellner J. **[Development of structure-activity relationships via topological indices]**. GSF Ber 1996;(12):88-94. (Ger)
CBAC COPYRIGHT: CHEM ABS Topol. indexes were used to det. quant. structure-activity relationships (QSAR) for alcs. and halogenated aliphates. The predicted toxicity of the chems. was compared to measured luminescent bacteria toxicity.

Cappelli A, Anzini M, Vomero S, De Benedetti PG, Menziani MC, Giorgi G, Manzoni C. **Mapping the peripheral benzodiazepine receptor binding site by conformationally restrained derivatives of 1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinolinecarb-oxamide (PK11195)**. J Med Chem 1997;40(18):2910-21.
CBAC COPYRIGHT: CHEM ABS A synthetic-computational approach to the study of the binding site of peripheral-benzodiazepine receptor (PBR) ligands related to 1-(2-chlorophenyl)-N-methyl-N-(1-

methylpropyl)-3-isoquinolinecarb- oxamide (PK11195; I) within their receptor has been developed. A wide series of conformationally restrained derivs. of I has been designed with the aim of probing the PBR binding site systematically. The synthesis of these compds. involves palladium-catalyzed coupling and amidation as the key steps. Twenty-nine rigid and semirigid derivs. of I were tested in binding studies using [3H]-I, and most of these showed PBR affinities in the nanomolar range. The essential role of the carbonyl moiety as a primary pharmacophoric element in the recognition by and the binding to PBR has been confirmed, and the restricted range of the carbonyl orientations, which characterizes the most potent ligands, points to a specific hydrogen-bonding interaction, mainly directed by the geometrical factors, when the electronic ones are fulfilled. Moreover, the fundamental importance of the short-range dispersive interactions in the modulation of the binding affinity and, hence, in the stabilization of the ligand-receptor complex, emerged from the QSAR models reported.

Chen H, Zhou J, Xie G, Ren T. **[QSAR research method based on pseudoreceptor model]**. Wuli Huaxue Xuebao 1997;13(7):626-31. (Chi)
CBAC COPYRIGHT: CHEM ABS In this paper, PARM algorithm which can be used in QSAR research was put forward. In this algorithm, a set of pseudo atom was defined and a series of pseudo receptor model was generated by using genetic algorithm. These models which have high correlation between receptor-ligand interaction and bioactivity can predict bioactivity of unknown mols. This algorithm was used to investigate the K+ channel opener system. The reasonable results were obtained.

De Paulis T, Hewlett W, Schmidt D, Mason N, Trivedi B, Ebert M. **Synthesis and 5-HT-3 receptor binding activity of 5-[125I]iodo-2,3-dimethoxy-N-(1-azabicyclo[2.2.2]oct-3-yl)benzamide and its 5-halogen-2-alkoxyl homologs**. Eur J Med Chem 1997;32(5):385-96.
CBAC COPYRIGHT: CHEM ABS Benzamide deriv serotonin receptor binding structure Azabicyclooctylbenzamide serotonin receptor binding structure Iodine labeling benzamide serotonin receptor binding PET iodine labeled benzamide serotonin receptor SPECT iodine labeled benzamide serotonin receptor;Lipophilicity Synthesis and 5-HT-3 receptor binding activity of [125I]iododimethoxy (1-azabicyclo[2.2.2]octyl)benzamide and 5-halogen alkoxyl homologs in relation to structure and use for PET and SPECT;Positron-emission tomography Synthesis and 5-HT-3 receptor binding activity of [125I] iododimethoxy(1-azabicyclo[2.2.2]octyl)benzamide and 5-halogen alkoxyl homologs in relation to structure and use for PET and SPECT;QSAR Synthesis and 5-HT-3 receptor binding activity of [125I] iododimethoxy(1-azabicyclo[2.2.2]octyl)benzamide and 5-halogen alkoxyl homologs in relation to structure and use for PET and SPECT Structure-activity relationship;Receptor-binding structure-activity relationship 5-HT3 receptor-binding; synthesis and 5-HT-3 receptor binding activity of [125I] iododimethoxy(1-azabicyclo[2.2.2]octyl)benzamide and 5-halogen alkoxyl homologs in relation to structure and use for PET and SPECT;Single-photon-emission computed tomography Synthesis and 5-HT-3 receptor binding activity of [125I]iododimethoxy(1-azabicyclo[2.2.2]octyl)benzamide and 5-halogen alkoxyl homologs in relation to structure and use for PET and SPECT;5-HT3 receptors Synthesis and 5-HT-3 receptor binding activity of [125I]iododimethoxy(1-azabicyclo[2.2.2]octyl) benzamide and 5-halogen alkoxyl homologs in relation to structure and use for PET and SPECT.

Do Amaral A, Oliveira A, Neidlein R, Gallacci M, Caprara L, Miyazaki Y. **Physicochemical parameters involved in the lethal toxicity of N,N-[(dimethylamino)ethyl]-4-substituted benzoate**

hydrochlorides: a QSAR study. Eur J Med Chem 1997;32(5):433-43.
CBAC COPYRIGHT: CHEM ABS A set of sixteen para-substituted N,N-[(dimethylamino)ethyl] benzoate hydrochlorides structurally related to procaine was synthesized. The apparent partition coeffs. were detd. by either shake-flask or HPLC methods and were taken as hydrophobic parameters. The IR stretching frequencies of the carbonyl group were detd. in chloroform and taken as one of the electronic parameters. Addnl. physicochem. parameters were either taken from the literature: pi, sigma, F and R, MR4, or calcd.: log P. The lethal potency was detd. in the mouse via the LD50. In order to verify the nature and the relative contributions of the physicochem. parameters to lethal toxicity, QSAR equations were derived using regression anal. A major contribution of hydrophobicity together with a smaller but still significant contribution of electronic or polar properties was found to describe the toxicity within this set of compds.

Easmon J, Heinisch G, Hofmann J, Langer T, Grunicke H, Fink J, Purstinger G. **Thiazolyl and benzothiazolyl hydrazones derived from alpha-(N)-acetylpyridines and diazines: synthesis, antiproliferative activity and CoMFA studies**. Eur J Med Chem 1997;32(5):397-408.
CBAC COPYRIGHT: CHEM ABS The synthesis of a series of thiazolyl and benzothiazolyl hydrazones derived from alpha-(N)-acylpyridines, -quinolines, -isoquinolines, -pyridazines, -pyrimidines, and -pyrazines is reported. The stereochem. of these compds. was detd. by NMR spectroscopic methods. The antiproliferative activity of the novel compds. was quantified in tissue culture (melanoma, breast carcinoma, colon adenocarcinoma, epitheloid cervix carcinoma, Burkitt's lymphoma, leukemia, and hydroxyurea sensitive and resistant myelogenous leukemia sublines). All compds. exhibited profound antiproliferative activity, in particular against Burkitt's lymphoma cells. Out of this series, some were 13-900 times more potent than hydroxyurea and no cross-resistance to hydroxyurea was obsd. A predictive 3D-QSAR model using the CoMFA approach was established.

Elguero J, Rozas I. **[Nonconventional analgesics: bradykinin antagonists]**. An R Acad Farm 1997;63 (1):173-90. (Spa)
CBAC COPYRIGHT: CHEM ABS A review with 34 refs. Bradykinin and kallidin, kinins, are generated by the activity of kallikreins (proteolytic enzymes) on kininogens. Kinins elicit pathophysiol. responses including pain and hyperalgesia. Kinins receptors are classified according to the relative potencies of agonist and antagonists. Regoli and Barabe proposed two subtypes of receptors, B1 and B2. Hundreds of agonists analogs of bradykinin were prepd. before the first antagonist compds. appeared. Synthetic efforts have been oriented towards peptidic analogs until few years ago when the search of non-peptidic antagonists started. The distribution of receptor B1 in the human being is very limited and probably this subtype plays an unimportant role on human diseases. Two generation of peptidic antagonists of the B2 receptor have been developed. The second generation has compds. two orders of magnitude more potent as analgesics than the first generation ones and the most important deriv. was icatibant. The first non-peptidic antagonist of the B2 receptor, described in 1993, has two phosphonium cations sepd. by a modified amino acid. Many derivs. of this dication have been prepd. Another non-peptidic compd. antagonist of B2 is the natural product Martinelline. Mol. modeling and QSAR studies have been carried out on bradykinin as well as on its antagonists.

Folkers G, Merz A. **Hydrophobic fields in quantitative structure-activity relationships**. Methods

Princ Med Chem 1996;4:219-32.
CBAC COPYRIGHT: CHEM ABS A review with 32 refs. Hydrophobic effects play a key role in the architecture of biopolymers and their interactions with small mols. Both the folding and the ligand interaction of proteins is predominantly governed by hydrophobicity. The same is true for building and stabilization of membranes, for instance by steroid-phospholipid interaction. Very recently, our attention has been drawn to a further, hitherto underestimated importance of hydrophobicity in protein-ligand interactions. DNA and RNA interaction with proteins might be more influenced by hydrophobic binding than it has been expected. Characterization of the TATA box recognition by its protein revealed that the nucleosides are nearly exclusively bound by hydrophobic interaction, and that this process is sufficient for a specific recognition. A more recent observation concerns the valine-binding part of RNA. The binding of the amino acid is quite specific, though it is merely hydrophobic. Even stereoisomers can be discriminated showing a 10- to 100-fold difference in affinity. In spite of the knowledge of many structural details, at least for cytosolic protein-ligand complexes, the nature of the hydrophobic effect has not yet been assessed exptl., nor formulated theor. in a satisfactory manner. Measurements and definitions of global mol. lipophilic properties like log P are valuable for quant. structure-activity relationships (QSAR) and drug formulation, but none of the approaches yield information about conformation dependency and mol. surface distribution of hydrophobicity. The later might be one of the most important point in the study of ligand-protein interactions and for the investigation of the biol. activity of drugs on mol. level. Therefore, an appropriate description of hydrophobicity at an at. level would be an important progress in drug design.

Gamage SA, Figgitt DP, Wojcik SJ, Ralph RK, Ransijn A, Mauel J, Yardley V, Snowdon D, Croft SL, Denny WA. **Structure-activity relationships for the antileishmanial and antitrypanosomal activities of 1'-substituted 9-anilinoacridines.** J Med Chem 1997;40(16):2634-42.
Members of the class of 9-anilinoacridine topoisomerase II inhibitors bearing lipophilic electron-donating 1'-anilino substituents are active against both the promastigote and amastigote forms of the parasite Leishmania major. A series of analogues of the known 1'-NHhexyl lead compound were prepared and evaluated against L. major in macrophage culture to further develop structure-activity relationships (SAR). Toxicity toward mammalian cells was measured in a human leukemia cell line, and the ratio of the two IC50 values (IC50(J)/IC50(L)) was used as a measure of the in vitro therapeutic index (IVTI). A 3,6-diNMe2 substitution pattern on the acridine greatly increased toxicity to L. major without altering mammalian toxicity, increasing IVTIs over that of the lead compound. The 2-OMe, 6-Cl acridine substitution pattern used in the antimalarial drug mepacrine also resulted in potent antileishmanial activity and high IVTIs. Earlier suggestions of the utility of 2'-OR groups in lowering mammalian cytotoxicity were not borne out in this wider study. A series of very lipophilic 1'-NRR (symmetric dialkylamino)-substituted analogues showed relatively high antileishmanial potency, but no clear trend was apparent across the series, and none were superior to the 1'-NH(CH2)5Me subclass. Subsets of the most active 1'-N(R)(CH2)5Me- and 1'-N(alkyl)2-substituted compounds against L. major were also evaluated against Leishmania donovani, Trypanosoma cruzi, and Trypanosoma brucei, but no consistent SAR could be discerned in these physiologically diverse test systems. The present study has confirmed earlier conclusions that lipophilic electron-donating groups at the 1'-position of 9-anilinoacridines provide high activity against L. major, but the SAR patterns observed do not carry over to the other parasites studied.

Hong H, Wang L, Zou G. **Retention in RP-HPLC: Lipophilicity determination of substituted biphenyls by reversed-phase high performance liquid chromatography.** J Liquid Chromatogr Rel Technol 1997;20(18):3029-37.
BIOSIS COPYRIGHT: BIOL ABS. The RP-HPLC capacity factors (k') of a series of substituted biphenyls were determined on a C18 column with methanol/water as the mobile phase. A linear relationship was found between log k' and the volume fraction of methanol (phi) for each of the 25 tested compounds with the correlation coefficient >0.99. High correlation was found between log k'w and S, which are intercept and slope of plot of log k'vs. phi, respectively. The values of S and log k'w were quantitatively correlated with the solvatochromic parameters of the solutes and two statistically significant equations were established which showed that solute volume and hydrogen bond basicity were of major importance in influencing the retention of these studied compounds. The obtained log k'w was chosen as a measure of the solute hydrophobicity and will be used for the on-going Quantitative Structure-Activity Relationship (QSAR) studies of the substituted biphenyls.

Jiang H, Chen K, Tang Y, Chen J, Li Q, Wang Q, Ji R. **Molecular modeling and 3D-QSAR studies on the interaction mechanism of tripeptidyl thrombin inhibitors with human alpha-thrombin**. J Med Chem 1997;40(19):3085-90.
CBAC COPYRIGHT: CHEM ABS The mechanism of inhibition of peptidyl inhibitors with thrombin was studied using mol. modeling, mol. mechanics, and CoMFA statistical anal. A new procedure for the elucidation of binding conformations, BCSPL, is described and was employed to obtain the binding conformers of a series of 18 tripeptidyl thrombin inhibitors. Energetic studies and QSAR anal. of the BCSPL-derived conformers indicated a modest correlation between the calcd. binding energies of the title compds. and their inhibitory activities to human alpha-thrombin. CoMFA anal. of the BCSPL alignment resulted in a satisfactory model of the thrombin active site.

Kochel B, Sajewicz W. **A new measure of xenobiotic toxicity to the first-line human defense system from the time-resolved phagocyte luminescence**. Bull Math Biol 1997;59(5):897-910.
CBAC COPYRIGHT: CHEM ABS A new measure of toxicity based on stochastic modeling of single photon-counting processes, representing time-resolved phagocyte luminescence of xenobiotic-perturbed human neutrophils, has been constructed. The stochastic measure of toxicity has been verified by the QSAR method, and then compared and contrasted with the traditional toxicity measure used in bio- and chemiluminescent research. Phenol and benzene homologs were chosen as perturbers due to their importance from the viewpoint of ecotoxicol. and occupational medicine.

Krylov SN, Huang XD, Zeiler LF, Dixon DG, Greenberg BM. **Mechanistic quantitative structure-activity relationship model for the photoinduced toxicity of polycyclic aromatic hydrocarbons: I. Physical model based on chemical kinetics in a two-compartment system.** Environ Toxicol Chem 1997;16(11):2283-95.
BIOSIS COPYRIGHT: BIOL ABS. A quantitative structure-activity relationship model for the photoinduced toxicity of 16 polycyclic aromatic hydrocarbons (PAHs) to duckweed (Lemna gibba) in simulated solar radiation (SSR) was developed. Lemna gibba was chosen for this study because toxicity could be considered in two compartments: water column and leaf tissue. Modeling of photoinduced

toxicity was described by photochemical reactions between PAHs and a hypothetical group of endogenous biomolecules (G) required for normal growth, with damage to G by PAHs and/or photomodified PAHs in SSR resulting in impaired growth. The reaction scheme includes photomodification of PAHs, uptake of PAHs into leaves, triplet-state formation of intact PAHs, photosensitization reactions that damage G, and reactions between photomodified PAHs and G. The assumptions used were: the PAH photomodification rate is slower than uptake of chemicals into leaves, the PAH concentration in aqueous solution is nearly constant during a toxicity test, the fluence rate of actinic radiation is lower within leaves than in the aqueous phase, and the toxicity of intact PAHs in the dark is negligible. A series of differential equations describing the reaction kinetics of intact and photomodified PAHs with G was derived. The resulting equation for PAH toxicity was a function of treatment period, initial PAH concentration, relative absorbance of SSR by each PAH, quantum yield for formation of triplet-state PAH, and rate of PAH photomodification. Data for growth in the presence of intact and photomodified PAHs were used to empirically solve for a photosensitization constant (PSC) and a photomodification constant (PMC) for each of the 16 PAHs tested. For 9 PAHs the PMC dominates and for 7 PAHs the PSC dominates.

Lahana R, McWilliams P, Holland J, Richards WG. **3D-QSAR analysis of the binding of triazine herbicides to a monoclonal antibody**. In: Van De Waterbeemd H, Testa B, Folkers G, Editors. Computer-Assisted Lead Finding and Optimization: Current Tools of Medicinal Chemistry. New York: Wiley-VCH; 1997. P. 463-71.
CBAC COPYRIGHT: CHEM ABS The use of a newly designed architecture has allowed us to apply many disparate algorithms to a diverse range of QSAR applications. We present the use of this method applied to the QSAR anal. of a set of triazine herbicides. Recent environmental studies have focused on the binding of these species to antibodies, as a means of monitoring the toxicol. effects. We have used our integrated algorithm to predict the binding of triazines to a monoclonal antibody, and offer an insight into the means of binding.

Lemmen C, Lengauer T. **Time-efficient flexible superposition of medium-sized molecules**. J Comput Aided Mol Des 1997;11(4):357-68.
CBAC COPYRIGHT: CHEM ABS The authors present an efficient algorithm for the structural alignment of medium-sized org. mols. The algorithm has been developed for applications in 3D QSAR and in receptor modeling. The method assumes one of the mols., the ref. ligand, to be presented in the conformation that it adopts inside the receptor pocket. The second mol., the test ligand, is considered to be flexible, and is assumed to be given in an arbitrary low-energy conformation. Ligand flexibility is modeled by decompg. the test ligand into mol. fragments, such that ring systems are completely contained in a single fragment. Conformations of fragments and torsional angles of single bonds are taken from a small finite set, which depends on the fragment and bond, resp. The algorithm superimposes a distinguished base fragment of the test ligand onto a suitable region of the ref. ligand and then attaches the remaining fragments of the test ligand in a step-by-step fashion. During this process, a scoring function is optimized that encompasses bonding terms and terms accounting for steric overlap as well as for similarity of chem. properties of both ligands. The algorithm has been implemented in the FLEXS system. To validate the quality of the produced results, the authors have selected a no. of examples for which the mutual superposition of two ligands is exptl. given by the

comparison of the binding geometries known from the crystal structures of their corresponding protein-ligand complexes. On more than two-thirds of the test examples the algorithm produces rms deviations of the predicted vs. the obsd. conformation of the test ligand below 1.5 .ANG.. The run time of the algorithm on a single problem instance is a few minutes on a common-day workstation. The overall goal of this research is to drastically reduce run times, while limiting the inaccuracies of the model and the computation to a tolerable level.

Matova M, Nacheva R, Boicheva S. **QSAR analysis of 2-alkyloxy and 2-aralkyloxy adenosine A1- and A2-agonists**. Eur J Med Chem 1997;32(6):505-13.
CBAC COPYRIGHT: CHEM ABS A quant. structure-activity relationship (QSAR) anal. of a series 2-alkyloxy-, 2-aryloxy- and 2-aralkyloxy-adenosines has been performed. Various theor. 3-D electronic and topol. descriptors encoding their mol. structure were estd. and the structure-activity correlations were evaluated. A cluster anal. of the affinity consts. of the compds. was carried out, and according to the obtained results the QSAR anal. was developed at two levels. The results of this investigation allowed a distinction to be made between A1- and A2-receptor selectivity of the compds. due to structural reasons. It was shown that small and less lipophilic substituents may enhance the A1-receptor selectivity of the compds. Hydrophobic and bulky cycloalkyl substituents greatly enhance A2-receptor selectivity. The more lipophilic and rigid arom. substituents increase the affinity, but decrease selectivity at both receptors. Adenosine agonist activity is also detd. by the electron-donating properties of the purine ring and of certain atoms in this arom. system: the N6 atom in A1-selective ligands and the N1, N7, C2, C5, C6, C8 atoms in A2-selective ligands appear to constitute part of the pharmacophore of the mols.

Mazzullo M, Mesirca R, Paolini M, Cantelli-Forti G, Perocco P, Ciaccia P, Grilli S. **A database for evaluating the toxicological risk of pesticides.** J Environ Pathol Toxicol Oncol 1997;16(2-3):231-7.
BIOSIS COPYRIGHT: BIOL ABS. This study of Overtox-DB, a computerized database for managing chemical toxicity data, is a product of the application of typical methodologies regarding information science and computer technology. The methodology applied can be reduced to three basic elements: the collection of requirements, design, and achievement. Overtox-DB was developed by defining technological elements for managing data and its structure and by identifying the procedures and methodologies for data storage, retrieval, distribution, and standardization of many kinds of test data stored in the same format. The program stores data about chemical identification, physical and chemical properties, toxicological tests, mutagenicity, teratogenicity, carcinogenicity, and a bibliography of chemical compounds. Overtox-DB consists of five modules: experimental and bibliographic, data collection, molecular data collection, data search, and data report. The Overtox-DB user responds to a simplified set of query commands and boolean operators that interact with the system to retrieve different toxicological data (the majority of fields are defined as search fields and identify the test system, results of the assays, administration route, dose, etc.). The collected information provides an analytical characterization of biological activities for many compounds and identifies evidence possibly lacking in experimental approaches. Indeed, this database could permit a comparative evaluation with other substances and can be used for structure-activity relationship studies.

Poelloth C, Mangelsdorf I. **Commentary on the application of (Q)SAR to the toxicological**

evaluation of existing chemicals. Chemosphere 1997;35(11):2525-42.
BIOSIS COPYRIGHT: BIOL ABS. For ethical and financial reasons it is impossible to perform thorough toxicological testing for all of the more than 100,000 substances registered in the European Inventory of Existing Substances. It was therefore investigated whether the application of (quantitative) structure-activity relationships (QSAR) with commercially available computer programs could predict the toxicological profile and help identify those substances requiring priority toxicological testing. Whereas predictions with respect to complex endpoints such as carcinogenicity, chronic toxicity and teratogenicity are still disappointing, more reliable predictions should be forthcoming in the immediate future for sensitisation, mutagenicity and genotoxicity endpoints.

Schultz TW, Sinks GD, Cronin MT. **Quinone-induced toxicity to Tetrahymena: structure-activity relationships.** Aquatic Toxicol 1997;39(3-4):267-78.
BIOSIS COPYRIGHT: BIOL ABS. The aquatic toxicities (log(IGC50-1)) of 22 quinones were evaluated in the static Tetrahymena pyriformis population growth assay. A toxicity in excess of baseline was associated with each chemical eliciting a response. The ortho-quinones were the most toxic; internal quinones, with benzenoid substitution on both sides of the quinoid rings, were not toxic at saturation. Ring substitution of 1,4-benzoquinone resulted in a graded toxic response. Substitution by electron-donating, methoxy or hydroxy groups decreased the toxicity. The position of ring substitution was also important, with 2,5-substitution being less toxic than 2,6-substitution. The fully substituted tetramethyl and tetrachloro derivatives, quinones which cannot conjugate or arylate protein thiols, were significantly less toxic than 1,4-benzoquinone. Due to their molecular structure, quinones may act as soft electrophiles and/or redox cyclers. This mixture of mechanisms of toxic action precludes high-quality, quantitative, structure-toxicity relationships. The toxic potency was found to be independent of the 1-octanol/water partition coefficient, one-electron redox potential and lowest unoccupied molecular orbital energy.

Shishoo CJ, Shirsath VS, Rathod IS, Brahmbhatt SB, Pathak US, Jain KS. **Synthesis and QSAR of some 3-amino-2-(substituted)aminomethyl-5,6-disubstituted thieno[2,3-d]pyrimidin-4(3H)-ones as novel H1-receptor antagonists**. Drug Des Discovery 1997;15(2):105-15.
CBAC COPYRIGHT: CHEM ABS The present study describes the synthesis and quant. structure activity relationships (QSAR) of novel 3-amino-2-(substituted)aminomethyl-5,6-disubstitutedthieno[2,3-d]- pyrimidin-4(3H)-ones for their potent H1-receptor antagonist activity on the guinea pig ileum. With the IC50 values in the range of 10-5 g/L, all the compds. tested were found to possess ten fold higher affinity to the H1-receptor than diphenhydramine and cetirizine, but lower than astemizole and loratidine. The sedative potential of these compds. was lower than cetirizine and astemizole but comparable to loratidine. The QSAR study indicates a parabolic relation of the biol. activity mainly with the steric parameters and partly with the lipophilic parameters.

Smith CJ, Hansch C, Morton MJ. **QSAR treatment of multiple toxicities: the mutagenicity and cytotoxicity of quinolines.** Mutat Res 1997;379(2):167-75.
BIOSIS COPYRIGHT: BIOL ABS. A series of 15 quinoline congeners were assayed for mutagenicity and cytotoxicity in the Ames test using strain TA100 bacteria. Statistical analysis of the data allowed simultaneous determination of the mutagenicity and cytotoxicity of each quinoline. These data were

used to develop three quantitative structure-activity relationships (QSAR). In all three QSAR, the strength of the relationship between hydrophobicity (as measured by log P) and biological activity was similar as h was near 1 in all three cases. For the mutagenicity of these quinolines, both hydrophobic and steric interactions appear to be important. In contrast, the cytotoxicity is mainly affected by increasing hydrophobicity and by the addition of electron withdrawing substituents to the quinoline ring. Comparison to other QSAR from our laboratory and others lends support to these findings. Both simultaneous consideration of different biological activities and the comparison of newly developed QSAR with previous data for the purpose of lateral validation should be encouraged in future QSAR studies.

Tatara CP, Newman MC, McCloskey JT, Williams PL. **Predicting relative metal toxicity with ion characteristics: Caenorhabditis elegans LC50.** Aquatic Toxicol 1997;39(3-4):279-90.
BIOSIS COPYRIGHT: BIOL ABS. Quantitative Structure Activity Relationships (QSAR) predict relative toxicity of a family of chemicals from fundamental and surrogate molecular qualities. Most QSARs are developed for organic toxicants, with inorganic toxicants (metals) being under-represented. Successful predictive models for relative toxicity of divalent metal ions using ion characteristics have been produced using Microtox, a 15 min microbial bioassay. The present study extends this approach to longer exposure durations (24 h), and a more complex organism (metazoan). Twenty-four hour LC50s (expressed as total metal concentration) for the free-living soil nematode, C elegans were determined for Ca, Cd, Cu, Hg, Mg, Mn, Ni, Pb, and Zn in an aqueous medium. Relative metal toxicity was predicted with least squares linear regression and several ion characteristics. Toxicity was most effectively predicted (r2 =0.89) with ! log KOH ! (where KOH is the first hydrolysis constant), which reflects a metal ion's tendency to bind to intermediate ligands such as biochemical functional groups with 0 donor atoms. The best fitting model was obtained using LC50 metameters based on total metal concentration, indicating that the identification of the bioactive species of metals can be ambiguous, and does not necessarily aid in the prediction of relative metal toxicity with ion characteristics. The modelling of relative metal toxicity using ion characteristics was successful for 24 h exposure durations using this more complex organism.

Tong W, Perkins R, Strelitz R, Collantes ER, Keenan S, Welsh WJ, Branham WS, Sheehan DM. **Quantitative structure-activity relationships (QSARs) for estrogen binding to the estrogen receptor: predictions across species.** Environ Health Perspect 1997;105(10):1116-24.
The recognition of adverse effects due to environmental endocrine disruptors in humans and wildlife has focused attention on the need for predictive tools to select the most likely estrogenic chemicals from a very large number of chemicals for subsequent screening and/or testing for potential environmental toxicity. A three-dimensional quantitative structure-activity relationship (QSAR) model using comparative molecular field analysis (CoMFA) was constructed based on relative binding affinity (RBA) data from an estrogen receptor (ER) binding assay using calf uterine cytosol. The model demonstrated significant correlation of the calculated steric and electrostatic fields with RBA and yielded predictions that agreed well with experimental values over the entire range of RBA values. Analysis of the CoMFA three-dimensional contour plots revealed a consistent picture of the structural features that are largely responsible for the observed variations in RBA. Importantly, we established a correlation between the predicted RBA values for calf ER and their actual RBA values for human ER.

These findings suggest a means to begin to construct a more comprehensive estrogen knowledge base by combining RBA assay data from multiple species in 3D-QSAR based predictive models, which could then be used to screen untested chemicals for their potential to bind to the ER. Another QSAR model was developed based on classical physicochemical descriptors generated using the CODESSA (Comprehensive Descriptors for Structural and Statistical Analysis) program. The predictive ability of the CoMFA model was superior to the corresponding CODESSA model.

Turner DB, Willett P, Ferguson AM, Heritage T. **Evaluation of a novel infrared range vibration-based descriptor (EVA) for QSAR studies. 1. General application**. J Comput Aided Mol Des 1997;11 (4):409-22.
CBAC COPYRIGHT: CHEM ABS A novel mol. descriptor (EVA) based upon calcd. IR range vibrational frequencies is evaluated for use in QSAR studies. The descriptor is invariant to both translation and rotation of the structures concerned. The method was applied to 11 QSAR datasets exhibiting both a range of biol. endpoints and various degrees of structural diversity. This study demonstrates that robust QSAR models can be obtained using the EVA descriptor and examines the effect of EVA parameter changes on these models; recommendations are made as to the appropriate choice of parameters. The performance of EVA was comparable in statistical terms to that of CoMFA, despite the fact that EVA does not require the generation of a structural alignment. Models derived using semiempirical (MOPAC AM1 and PM3) and AMBER mechanics calcd. normal mode frequencies are compared, with the overall conclusion that the semiempirical methods perform equally well and both outperform the AMBER-based models.

Wang T, Zhou J. **[Evolutionary algorithm as a strategy for variable selection in QSAR studies]**. Jisuanji Yu Yingyong Huaxue 1997;14(2):95-100. (Chi)
CBAC COPYRIGHT: CHEM ABS Application to literature data on larvicides and sulfonylurea herbicides demonstrates that the evolutionary algorithm is an effective tool for variable selection and building multiple QSAR models. An appropriate fitness function used for evaluating the models is a key to obtaining high quality models.

Zhang YP, Sussman N, Klopman G, Rosenkranz HS. **Development of methods to ascertain the predictivity and consistency of SAR models: application to the U.S. National Toxicology Program rodent carcinogenicity bioassays.** Quant Struct Activity Relat 1997;16(4):290-5.
BIOSIS COPYRIGHT: BIOL ABS. Models investigating relationships between chemical structures and biological activities are receiving increased recognition for the identification of chemicals with the potential for inducing adverse health effects. The relationships can be either qualitative (noted as SAR) or quantitative (noted as QSAR). The objective of the present study was to define an effective process for evaluating such models. The predictivity of SAR/QSAR models derived from the U.S. National Toxicology Program Rodent Carcinogenicity Bioassay endeavor by CASE/MultiCASE was evaluated by several different approaches: leave-one-out tests, 10-fold cross-validations and by the use of an independent test set. The goodness-of-fit for the data used in the model building, the predictivity for the chemicals not contained in the model, and the consistency of the predictions for a group of chemicals by different SAR/QSAR sub-models were examined systematically. Individual prediction indices generated by CASE/MultiCASE, arbitrary combinations thereof, as well as weighted combinations using Bayes'

theorem, were utilized to derive predictions of carcinogenicity. Combinations derived using Bayes' theorem provided the most predictive model. The closeness between sub-models based on the leave-one-out procedure and the full model (all chemicals used for model building) makes it the most reliable process for the estimation of a model's predictivity. However, the similarity between the predictions of the leave-one-out models and the 10-fold cross-validation models indicates that the latter process provides an acceptable approach.

Zhu Y, Yu Y, Chen X. **[Fisher discriminant analysis of structure-carcinogenicity relationship of polycyclic aromatic hydrocarbons]**. Weish Dulixue Zazhi 1997;11(2):71-4. (Chi)
CBAC COPYRIGHT: CHEM ABS The Fisher discriminant anal. of structure-carcinogenicity relation of polycyclic arom. hydrocarbons was carried out using physiochem. parameters and/or structure parameters. The results indicated that available physiochem. parameters could be applied only to specific chem. class such as N-nitroso compds. There is no significant difference of discriminant performance between the optimized computer automated structure evaluation (CASE) and this study using structure parameters alone or combined with physiochem. ones.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Aravindan GR, Bjordahl J, Jost LK, Evenson DP. **Susceptibility of human sperm to in situ DNA denaturation is strongly correlated with DNA strand breaks identified by single-cell electrophoresis.** Exp Cell Res 1997;236(1):231-7.
Susceptibility of mammalian sperm DNA to low pH- or heat-induced denaturation in situ has shown very strong dose-response relationships with animal and human exposure to chemical and physical toxicants and also fertility potential. In this study, 23 human semen samples representing a wide range in percentage (7-86%) of sperm exhibiting abnormally high susceptibility of DNA in situ to denaturation were studied for the integrity of their DNA using alkaline comet assay (single-cell microgel electrophoresis, pH 10.0). The percentage of comets observed for these samples ranged from 5 to 95%; these data correlated strongly with the percentage of sperm with increased DNA denaturability ($r = 0.973$; $P < 0.001$). Labeling of 3' ends of nicked DNA sites with 5-bromo-2'-deoxyuridine 5'-triphosphate (BrdUTP) followed by tagging with FITC-BrdUTP monoclonal antibody and flow cytometry also indicated significantly strong correlations of BrdUTP incorporation with both abnormal susceptibility of DNA to denaturation ($r = 0.859$, $P < 0.001$) and comet assay ($r = 0.812$, $P < 0.001$). The relationship among susceptibility of sperm chromatin to acid denaturation in situ, BrdUTP incorporation, and formation of comets suggests that DNA fragmentation monitored by these assays may have important physiological relevance in terms of sperm quality and fertility potential.

Carney EW, Hoberman AM, Farmer DR, Kapp RW Jr, Nikiforov AI, Bernstein M, Hurtt ME, Breslin WJ, Cagen SZ, Daston GP. **Estrogen modulation: tiered testing for human hazard evaluation. American Industrial Health Council, Reproductive and Developmental Effects Subcommittee.** Reprod Toxicol 1997;11(6):879-92.
Recent concerns about the potential of certain chemicals to modulate estrogen-regulated processes have led to questions as to how chemicals should be tested for such effects. Therefore, AIHC has developed a comprehensive, resource-efficient, and flexible tiered strategy for estrogen modulation (EM) testing.

Levels of evaluation include Tier 0, in which exposure, along with alerts based on structure-activity, persistence, bioaccumulation, and other data, are assessed to prioritize chemicals for preliminary testing. In Tier I, short term in vitro, ex vivo, and/or in vivo assays are used to obtain a preliminary indication of EM potential. Among these, an in vivo response assay is considered the most reliable at this time. However, none of these tests are intended for risk assessment, but rather to aid in choosing chemicals for further testing and in guiding the extent of that testing. Tier II is aimed at risk assessment and involves whole animal tests that contain EM-sensitive end points (e.g., two-generation reproduction study). Tier III consists of hypothesis-driven research reserved for situations where targeted research can reduce levels of uncertainty. This tiered approach provides a framework for the strategic and effective application of EM test methods to address specific information needs on a case by case basis.

Clerici LA, Cocco B, Sacco MG, Monteggia E, Collotta A. **The use of pre-implantation mouse embryos cultured in vitro in toxicological studies**. Toxicol In Vitro 1995;9(5):577-81.
Studies were performed to evaluate the embryotoxic properties of tritium (10028178) and 1,2:3,4-diepoxybutene (DEB) using preimplantation mouse embryos cultured in-vitro. Exposures of blastocysts for 24 hours to concentrations up to 0.296 kilobecquerels/milliliter tritiated amino acids or nucleoside induced a statistically significant reduction in the percentage of embryos that reached the stage of two layer inner cell mass (ICM). The same quantity of tritiated arginine, but not of tritiated thymidine or tritiated tryptophan, also induced a lower percentage of differentiating ICM than the control when added to culture medium during the second cleavage division. DEB was highly embryotoxic in preimplantation mouse embryos at micromolar concentrations. The most sensitive stages of preimplantation development were found to be the two and four cell embryos. These results were consistent with in-vivo reports in the literature. The authors conclude that the results support the idea that tritium released by nuclear powerplants could become a radiotoxicological problem since it can be converted easily into organic compounds by living organisms. Further studies are needed on the embryotoxic effects of DEB.

Cook JC, Kaplan MA, Davis LG, O'Connor JC. **Development of a Tier I screening battery for detecting endocrine-active compounds (EACs).** Regul Toxicol Pharmacol 1997;26(1 Pt 1):60-8.
One of the components of our research program is development of a mode-of-action screening battery to detect several different types of endocrine-active compounds (EACs). Our working hypothesis is that a comprehensive short-term in vivo/in vitro battery can be developed to identify endocrine toxicants using a collection of endpoints. The goals of this battery are that it be quick, cost effective, and predictive. The purpose of this battery is to identify potential EACs and to assess their potency in order to prioritize compounds for further study. Two in vivo screens (intact male and ovariectomized female rats) are being evaluated for their ability to detect several different types of endocrine activity. To validate this screen, 15 compounds with known endocrine activities are being used to evaluate a collection of different endpoints for their variability, stability over time, predictiveness, and dose dependency. These positive controls were chosen because they can modulate development, reproduction, or cancer. The advantage of an in vivo screen is that it utilizes a metabolically and physiologically intact system. The male in vivo battery will be used to assess several different types of endocrine activity, primarily by using a comprehensive hormonal battery. The female in vivo battery will be used to identify compounds which are either estrogenic/antiestrogenic or can alter the prolactin pathway. The in vitro portion of the screening battery consists of a yeast transactivation system (YTS). The YTS is being evaluated for its

ability to identify compounds which are agonists or antagonists to the estrogen, androgen, or progesterone receptors. The expression of mammalian receptors in yeast allows for assessment of steroid-dependent transcriptional activators. The value of this system is that it can be used as a routine screen for compounds that interact with steroid receptors. Alterations in ligand binding to these receptors can be correlated with alterations in development via masculinization of females and/or feminization of males, decreases in reproductive success, or modulation of cancer incidence from in vivo tests. The in vivo and in vitro screens are designed to be run in parallel with built-in redundancy in order to reduce the probability of false-negative/ positive responses.

Fadool JM, Brockerhoff SE, Hyatt GA, Dowling JE. **Mutations affecting eye morphology in the developing zebrafish (Danio rerio).** Dev Genet 1997;20(3):288-95.
The zebrafish (Danio rerio) has received considerable attention as a mainstream model for the molecular and genetic study of vertebrate development. In our laboratory, we have conducted a third-generation screen of chemically mutagenized zebrafish for recessive mutations affecting the visual system. This report describes the visible phenotypes and number of morphological mutants so far observed and presents a more detailed histological analysis of six of these mutations. Through analysis of mutant larvae, it was determined that several of the subtle morphological mutations resulted in degeneration of specific cellular layers of the retina. Other mutations resulted in some degeneration distributed diffusely across the entire retina or concentrated at the retinal margin. A single mutation affecting invagination of the optic cup and lens vesicle formation resulted in a failure to develop an anterior chamber. These results demonstrate the utility of a small-scale, highly focused screen for uncovering novel loci involved in retinal and eye development.

Fort DJ, Stover EL. **Significance of experimental design in evaluating ecological hazards of sediments/soils to amphibian species**. ASTM Spec Tech Publ 1997;1317:427-42.
CBAC COPYRIGHT: CHEM ABS In an effort to det. the significance of exptl. design on the results of lab. sediment toxicity studies with amphibians (Xenopus laevis), 2 different sample prepns. were evaluated from 3 different contaminated waste sites. Whole sediment and aq. sediment exts. from each site were evaluated. Site 1 soil was characterized as loamy with a relatively high total org. carbon (TOC), moisture fraction (MF), and sulfide content; and contaminated with organochlorine pesticides. Site 2 soil was characterized as silty/clay with low/moderate TOC, MF, and sulfide; and contaminated with polynuclear arom. hydrocarbons (PAHs) and pentachlorophenol. Site 3 soil samples consisted of 2 sep. subsamples, the first characterized as loamy with a relatively high TOC, MF, and sulfide content, and the second as a mixt. of silty/clay and sand with relatively low TOC, MF, and sulfide content. Both subsite samples were contaminated with heavy metals, including copper, lead, and zinc. FETAX (Frog Embryo Teratogenesis Assay - Xenopus) testing of Site 1 samples indicated that substantially greater levels of developmental toxicity were induced by the aq. exts. than the whole bulk soil. Tests with Site 2 samples suggested that both of the prepns. were capable of inducing comparable rates of developmental toxicity. Tests with subsample a of Site 3 indicated that the aq. ext. of the sample induced greater levels of developmental toxicity than the whole soil. Toxicity tests with subsample b produced variable results that seemed to suggest that the exts. induced greater levels of toxicity than the whole bulk prepns. However, the differences in toxicities noted between the 2 prepns. were not as dramatic as obsd. in the Site 3a subsample tests. Results from these studies suggested the importance of exptl. design in

evaluating potential ecol. hazards of contaminated sediments or soils, particularly to amphibian species.

Friedman M, Burns CF, Butchko CA, Blankemeyer JT. **Folic acid protects against potato glycoalkaloid alpha-chaconine-induced disruption of frog embryo cell membranes and developmental toxicity.** J Agric Food Chem 1997;45(10):3991-4.
BIOSIS COPYRIGHT: BIOL ABS. To demonstrate whether folic acid can protect Xenopus embryos against reported adverse effects of the potato glycoalkaloid alpha-chaconine, the frog embryos were exposed simultaneously to the glycoalkaloid, folic acid (pteroylglutamic acid), and an electrochromic fluorescent dye, Di-4-ANEPPS, in a specially designed instrument that measures embryonic membrane potential. Folic acid decreased the chaconine-induced fluorescence, with a maximum decrease occurring at about 10 mg/L of both folic acid and the glycoalkaloid dissolved in solution. The protective effect was also operative in the frog embryo teratogenesis assay-Xenopus (FETAX), in which survival and teratogenicity of the whole embryos were the endpoints. Possible mechanisms of the protective effect and the possible significance of the results to food safety and health are discussed.

Henshel DS, Sparks DW, Mayer C A, Benson K, Fox C, Lam Y, Sobiech SA, Wagey R. **Preliminary results using early embryo teratogenesis assay: a comparison of early embryo abnormalities with late ambryo and hatchling teratogenic changes.** ASTM Spec Tech Publ 1997;1317:391-401.
BIOSIS COPYRIGHT: BIOL ABS. RRM BOOK CHAPTER MEETING PAPER CHICKEN SWALLOW BIRD HATCHLING NESTLING EMBRYO TOXICOLOGY EMBRYO TERATOGENESIS 2 3 7 8-TETRACHLORODIBENZO-P-DIOXIN EMBRYOTOXIN TERATOGENS TCDD ENVIRONMENTAL CONTAMINANTS DEVELOPMENT ECOLOGICAL IMPACT ASSESSMENT METHODOLOGY.

Hughes CM, Lewis SE, McKelvey-Martin VJ, Thompson W. **A comparison of baseline and induced DNA damage in human spermatozoa from fertile and infertile men, using a modified comet assay.** Mol Hum Reprod 1996;2(8):613-9.
Baseline DNA damage in spermatozoa from fertile and infertile men was compared using a modified alkali single cell gel electrophoresis (comet) assay. Semen from normozoospermic fertile, normozoospermic infertile and asthenozoospermic infertile (World Health Organization criteria, 1992) samples were studied. No significant difference was observed in levels of baseline damage between the three groups. A median value for baseline damage of approximately 20% (80% head DNA) was obtained in all samples. Irradiation with X-rays (5-30 Gy) produced no additional damage in fertile samples when median values were examined. However, irradiation with 30 Gy X-rays produced significant damage in both infertile groups. Hydrogen peroxide (40 microM) treatment induced significant damage in the asthenozoospermic group, whereas 100 microM H2O2 was required to cause significant damage in the normozoospermic fertile and infertile samples. Within the fertile population a subgroup in which percentage head DNA was greater than 80% was observed in both treated and untreated specimens. This subgroup significantly decreased with treatment in both infertile groups. We conclude that the asthenozoospermic infertile group is more susceptible to damage than the normozoospermic infertile group, which in turn is more susceptible than the fertile group. The fertile group contains a resistant subpopulation of spermatozoa with relatively intact DNA.

Kanti A, Smith MA. **Effects of heavy metals on chondrogenic differentiation of embryonic chick limb cells.** In Vitro Toxicol 1997;10(3):329-38.
BIOSIS COPYRIGHT: BIOL ABS. Despite evidence that environmental contaminants affect growth and development and that good in vitro models exist for investigating normal cartilage development and bone formation (Puzas et al., 1992), there is very little information on the effects of environmental pollutants on these systems. Five heavy metals, arsenic (As), cadmium (Cd), chromium (Cr), methyl mercury (methyl Hg), and lead (Pb), were investigated for their effects on chondrogenesis in an in vitro chick limb micromass culture system. Confluent monolayer cultures were prepared from chick distal wing tips, incubated for 120 hours, fixed and stained for proteoglycans and cell proliferation. Photomicrographs show qualitative differences in staining intensity between each metal and control cultures. Proteoglycan content as measured by Alcian green staining and cell proliferation as measured by Crystal Violet staining were determined spectrophometrically. Cadmium and methyl Hg at concentrations of 5 mug/ml and As, Cr, and Pb at concentrations of 50 mug/ml decreased proteoglycan content ($p < 0.05$) in chick limb micromass cultures. In temporal experiments, a decrease in proteoglycan content for As, Cd, Cr, methyl Hg, and Pb and a concomitant decrease for cell proliferation for As, Cd, Cr, and methyl Hg was seen by 120 hours of culture. In contrast, Pb (50mug/ml) showed a recovery in cell proliferation without a recovery in proteoglycan content, suggesting a specific lead-induced effect on chondrogenesis.

Lemly AD. **A teratogenic deformity index for evaluating impacts of selenium on fish populations.** Ecotoxicol Environ Safety 1997;37(3):259-66.
BIOSIS COPYRIGHT: BIOL ABS. This paper describes a method for using teratogenic deformities in fish as the basis for evaluating impacts of selenium contamination. Teratogenic deformities are reliable bioindicators of selenium toxicosis in fish. They are produced in response to dietary exposure of parent fish and subsequent deposition of selenium in eggs. There is a close parallel between selenium concentrations in eggs, incidence of teratogenic deformities in larvae, and magnitude of reproductive failure. Using these relationships, an index was developed for teratogenic-based assessment of impacts to fish populations. The index is composed of three ratings that signify increasing levels of terata-induced population mortality: 1, negligible impact (<5% population mortality); 2, slight to moderate impact (5-20% population mortality); 3, major impact (>20% population mortality). Each rating is based on the anticipated population-level impact of the corresponding degree of mortality. Teratogenic-based impact assessment provides a conclusive cause-effect linkage between the contaminant and the fish. It is particularly useful for verifying selenium-induced impacts on reproductive success because poor reproduction can be caused by many things - i.e., fluctuating water levels, nest predation, food shortages, poor recruitment, etc. The index given here should be a useful tool for evaluating the effect of selenium on fish populations. Moreover, application of this technique may save considerable time and money by identifying the most efficient use of man-power and funds early in the assessment process.

Luecke RH, Wosilait WD, Young JF. **Mathematical analysis for teratogenic sensitivity.** Teratology 1997;55(6):373-80.
BIOSIS COPYRIGHT: BIOL ABS. A mathematical structure is described for determining teratogenic sensitivity or susceptibility from analysis of malformation incidence, dose-response, and pharmacokinetic data obtained during pregnancy as a result of exposure to a teratogenic agent. From the

dosage or exposure of laboratory animals, embryonic and maternal concentrations of the xenobiotic are calculated using a physiologically based pharmacokinetic (PBPK) model. Malformations observed in the progeny are linked to the PBPK-derived target tissue concentrations with a model for the sensitivity calculated as a function of the embryonic age. The PBPK model for internal disposition of chemicals during pregnancy was developed previously. This report focuses on the development of the mathematical relations for the sensitivity of the embryo and effect functions on different organs. The concentrations of a xenobiotic calculated far the site of action or target tissue(s) in the embryo are weighted using both a nonlinear dose-response curve and a sensitivity distribution function that depends on the age or stage of development of the embryo. This weighted exposure of the target tissue is regressed with the number of observed malformations to quantify the parameters of the model. This approach lends itself to integration of diverse sources of experimental data, with hydroxyurea data taken from several sources in the literature as an example. This sensitivity function obtained from laboratory animal data serves as a vehicle for prediction and extrapolation to human pregnancy for the teratogenic potential of a substance.

Oberemm A, Fastner J, Steinberg C E. **Effects of microcystin-LR and cyanobacterial crude extracts on embryo-larval development of zebrafish (Danio rerio).** Water Res 1997;31(11):2918-21.
BIOSIS COPYRIGHT: BIOL ABS. Eggs of zebrafish (Danio rerio) were exposed to microcystin-LR at concentrations of 0.5, 5 and 50 mug litre-1 from blastula stage up to the end of embryonic development. No effects were observed during this period. After termination of exposure and transfer into microcystin-LR-free water, the evaluation at the end of the larval period showed decreased survival at 5 and 50 mug litre-1 microcystin-LR. Furthermore, concentrations of 0.5, 5 and 50 mug litre-1 microcystin-LR retarded larval growth. Far more pronounced effects occurred following exposure with various aqueous cyanobacterial crude extracts from field samples and batch cultures: gross malformations and high mortalities during embryonic development were observed.

Smith MA, Kanti A. **Chick embryo limb bud cell culture for screening environmental contaminants**. ASTM Spec Tech Publ 1997;1317:402-11.
CBAC COPYRIGHT: CHEM ABS Few non-mammalian systems have been used as models for assessing the developmental toxicity of environmental contaminants although the U.S. Environmental Protection Agency recognizes mammalian in vitro systems as appropriate developmental toxicity screens. The chick embryo micromass cell culture system was tested for its predictability to screen developmental toxicants to the skeletal system. Four toxicants with known toxicities in the rodent limb bud cell culture system (arsenate, aspirin, caffeine and methylmercury) were chosen for testing in the chick culture system. Cartilage-specific product, proteoglycans, were used to det. differentiation of the cells in culture by staining with Alcian Green then measured using a spectrophotometric method. Proliferation was detd. by staining with Crystal Violet. Dose and temporal response expts. were conducted to det. the most sensitive dose and time of exposure to cells in culture. Overall, the micromass cultures of the chick limb buds responded in a manner similar to those seen in the rodent culture.

Tilner J, Winckler T, Dingermann T. **Developmentally regulated promoters from Dictyostelium discoideum as molecular markers for testing potential teratogens**. Pharmazie 1996 Nov;51:902-6.

Ward KW, Blumenthal GM, Welsch F, Pollack GM. **Development of a physiologically based pharmacokinetic model to describe the disposition of methanol in pregnant rats and mice**. Toxicol Appl Pharmacol 1997;145(2):311-22.
CBAC COPYRIGHT: CHEM ABS The physiol. based pharmacokinetic (PBPK) model approach was used to develop a model of methanol disposition during gestation in rats and mice. To validate this model, concns. of methanol in the dam and the conceptus were detd. after methanol exposure of rats on Gestational Day (gd) 14 and 20 and of mice on gd 18. At the developmental stages examd., the model provided a good description of methanol disposition in the maternal circulation and the conceptus of both species. Furthermore, the model was capable of providing good fits to methanol concn.-time data from the literature. In pregnant animals, conceptal/maternal AUC and Cmax ratios decreased with increasing dose at both gd 14 and gd 20 in the rat and at gd 18 in the mouse. Addnl., the conceptal/ maternal diffusion const. ratio consistently decreased with increasing dose in pregnant rats and mice. These results are consistent with earlier observations that methanol limits its own delivery to the conceptus. Further experimentation is required to continue the process of developing a generalized PBPK model to describe the disposition of xenobiotics in pregnancy, to examine specific mechanisms of nonlinear conceptal methanol disposition, and to expand the model to extrapolate to low-dose human exposures.

Wiese TE, Polin LA, Palomino E, Brooks SC. **Induction of the estrogen specific mitogenic response of MCF-7 cells by selected analogs of estradiol-17beta: a 3D QSAR study**. J Med Chem 1997;40 (22):3659-69.
CBAC COPYRIGHT: CHEM ABS Analogs of estradiol-17beta (E2) have been evaluated for estrogen receptor (ER) binding affinity and mitogenic potential in the human breast cancer cell line MCF-7. These 42 compds. represent subtle modifications of the natural estrogen structure through the placement of hydroxyl, amino, nitro, or iodo groups around the ring system in addn. to, or as replacement of, the 3- and 17beta-hydroxyls of E2. The mitogenic activity of the analogs was found to be related to ER binding only to a limited extent. To elucidate structural features that are uniquely responsible for receptor binding affinity or mitogen potential of estrogens, the three-dimensional quant. structure-activity (QSAR) method Comparative Mol. Field Anal. (CoMFA) was employed. Sep. CoMFA models for receptor binding and cell growth stimulation were optimized through the use of various alignment rules and region step size. Whereas the CoMFA contour plots did outline the shared structural requirements for the two measured biol. properties, specific topol. features in this set of estrogens were delineated that distinguish mitogenic potential from ER binding ability. In particular, steric interference zones which affected growth extend in a band from above the A-ring to position 4 and below, whereas the ER binding steric interference zones are limited to isolated polyhedra in the 1,2 and 4 positions and the alpha face of the B-ring. In addn., electroneg. features located around the A-, B-, or C-rings contribute to receptor affinity. However, growth is dependent only on electroneg. and electropos. properties near the 3-position. In a final QSAR model for the mitogenic response, the value of ER binding was included along with structural features as a descriptor in CoMFA. The resulting 3D-QSAR has the most predictive potential of the models in this study and can be considered a prototype model for the general evaluation of a steroidal estrogen's growth stimulating ability in MCF-7 cells. For example, the location of D-ring contours illustrate the model's preference for 17beta-hydroxy steroids over the less mitogenic 17alpha- and 16alpha-hydroxy compds. In addn., the enhanced mitogenic effect of steric bulk in the 11alpha-

position is also evident. The QSAR studies in this report illustrate the fact that while ER binding may be a required factor of the estrogen dependent growth response in MCF-7 cells, particular structural characteristics, in addn. to those responsible for tight receptor binding, must be present to induce an optimal mitogenic response. Therefore, this report demonstrates that the CoMFA QSAR method can be utilized to characterize structural features of test compds. that account for different types of estrogenic responses.