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

A Bibliography with Abstracts

TO ASSIST IN:

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

PREPARED BY

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Vera W. Hudson, M.S.
Project Coordinator and Scientific Editor
National Library of Medicine

Elizabeth DeBerry
Copy Editor
National Library of Medicine

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.

National Library of Medicine, NIH
Specialized Information Services
Office of Hazardous Substance Information
2 Democracy Plaza, Suite 510
6707 Democracy Blvd., MSC 5467
Bethesda, MD 20892-5467 USA
Telephone: (301) 496-1131
FAX: (301) 480-3537

Email: Vera_Hudson@OCCSHOST.NLM.NIH.GOV

Suggestions and comments are welcome.

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CARCINOGENESIS

Afshar CE, Carrell CJ, Carrell HL, Harvey RG, Kiselyov AS, Amin S, Glusker JP. **Bay-region distortions in a methanol adduct of a bay-region diol epoxide of the carcinogen 5-methylchrysene**. Carcinogenesis 1996;17(11):2507-11.
BIOSIS COPYRIGHT: BIOL ABS. The three-dimensional structure of the product of the reaction of a diol epoxide of the carcinogen 5-methylchrysene with methanol has been determined by an X-ray diffraction analysis. The diol epoxide used to obtain this compound contains a stereochemically hindered bay region because of the location of the 5-methyl group, and this might be expected to affect the type of chemical reaction that occurs. The crystal structure analysis of this adduct of a polycyclic aromatic hydrocarbon (PAH) showed that a methoxy group has been added at the carbon atom of the epoxy group that is nearest to the aromatic system. The bond that is formed is axial to the ring.

Albert RE, French JE, Maronpot R, Spalding J, Tennant R. **Mechanism of skin tumorigenesis by contact sensitizers: The effect of the corticosteroid fluocinolone acetonide on inflammation and tumor induction by 2,4 dinitro-1-fluorobenzene in the skin of the TG.AC (v-Ha-ras) mouse.** Environ Health Perspect 1996;104(10):1062-8.
BIOSIS COPYRIGHT: BIOL ABS. The effect of the corticosteroid fluocinolone acetonide (FA) on skin tumor induction and inflammation by the contact sensitizer dinitrofluorobenzene (DNFB) was examined. This study broadly relates to the question of whether contact sensitizers, as electrophilic chemicals that produce protein adduction, may constitute an environmental cancer hazard. The specific aim of this study was to evaluate the extent to which the immunogenic inflammatory response to DNFB, in contrast to DNFB cytotoxicity, might be responsible for tumor induction. Experiments were conducted on a transgenic (TG.AC) mouse, incorporating a mutated ras oncogene (v-Ha-ras) that responds rapidly and profusely with skin papillomas to tumor promoters as if it were genetically initiated. Various doses and patterns of DNFB and FA were applied to the skin in a 2-week period; DNFB was given four times and FA was given either with the DNFB or daily. The tumor response to DNFB was completed by 8 weeks from the first dose and was consistent with a dose-squared relationship. FA was not tumorigenic alone; when given with DNFB; it caused only a small reduction in inflammation and tumor yield. When given daily, FA increased ulcerative skin damage, inflammation, and the yield of tumors. The results suggest that tumorigenesis by DNFB, in the high-dose short-term regimen used here, is mainly due to its cytotoxicity and not contact sensitization.

Arif JM, Gupta RC. **Detection of DNA-reactive metabolites in serum and their tissue distribution in mice exposed to multiple doses of carcinogen mixtures: role in human biomonitoring.** Carcinogenesis 1996;17(10):2213-9.
Our previous studies suggested that body fluids such as serum can serve as a novel surrogate for DNA-containing tissues to overcome the major limiting factor of tissue availability in human biomonitoring assessments. We have now examined the detectability of DNA-reactive metabolites (DRMs) in the

serum and tissues of male C57BL/6 mice administered up to 10 i.p. injections on alternate days of individual polynuclear aromatic hydrocarbons.

Ashby J. **Alternatives to the 2-species bioassay for the identification of potential human carcinogens**. Hum Exp Toxicol 1996;15(3):183-202.
It is proposed that the standard 2-species rodent cancer bioassay protocol, as perfected by the US National Toxicology Program (NTP), has already fulfilled its most useful role by providing an unequalled carcinogenicity database by which to re-assess the type of carcinogen worthy of definition. Continued use of this resource and time consuming protocol can no longer be justified, except in rare circumstances of high and protracted human exposure to a chemical of unknown carcinogenicity. In those rare instances an enlarged bioassay of three or four test species should perhaps be considered, there being nothing fundamental about the rat/mouse combination. In the large majority of cases, however, a practical estimation of the carcinogenic potential of a chemical can be formed in the absence of lifetime carcinogenicity bioassay data. This can be achieved by its sequential study, starting with an appreciation of its chemical structure and anticipated reactivity and mammalian metabolism. After the shortterm evaluation of a range of additional properties of the agent, including its genetic toxicity, rodent toxicity and tissue-specific toxicity, confident predictions of the genotoxic and/or non-genotoxic carcinogenic potential of the agent can be made. In most situations these predictions will be suitable for framing hazard reduction measures among exposed humans. In some situations it may be necessary to evaluate these predicted activities using limited bioassays, a range of which are considered. Extensions of these limited carcinogenicity bioassays to a standard 2-year/2-species bioassay can only be supported in cases where the non-carcinogenicity of the agent becomes the important thing to define. The US NTP have evaluated the carcinogenicity of approximately 400 chemicals over the past 20 years, at a cost of hundreds of millions of US dollars. The experience gained by that and related initiatives, worldwide, can now be harnessed to classify thousands of priority chemicals as being either probable carcinogens or probable noncarcinogens. That can now be achieved using a fraction of the earlier resources and in a fraction of the time that would be required for the conduct of 2-species bioassays. The comfort factor for one group of people of the order of the present system, coupled to the comfort factor for another group of the delay in carcinogenicity assessment enforced by the present council of perfection, are the two main factors delaying transfer to a streamlined system for assessing the carcinogenic potential of chemicals to humans. A third delaying factor in the need for new and focused test data. Coordinated acquisition of such data could rapidly remove the first two obstacles.

Ashby J, Kier L, Wilson AG, Green T, Lefevre PA, Tinwell H, Willis GA, Heydens WF, Clapp MJ. **Evaluation of the potential carcinogenicity and genetic toxicity to humans of the herbicide acetochlor**. Hum Exp Toxicol 1996;15(9):702-35.
Comprehensive toxicological studies of the herbicide acetochlor are presented and discussed. Although it gave a negative profile of responses in the many toxicity tests conducted there were some findings that prompted further investigation. First, although non-mutagenic in the Salmonella assay, acetochlor was clastogenic to mammalian cells treated in vitro. This clastogenic potential was not expressed in vivo in four rodent cytogenetic assays (bone marrow and germ cells). Second, although acetochlor gave a negative response in rat liver UDS assays when tested at the acute MTD, gavage administration of a single, supra-MTD dose (2000 mg/kg) gave a weak positive assay response. This dose-level (2000 mg/

kg) was necrotic to the liver, depressed hepatic glutathione levels by up to approximately 80%, altered the metabolism of acetochlor, and was associated with up to 33% lethality. In contrast, reference liver genotoxins such as DMN, DMH and 2AAF were shown to elicit UDS in the absence of such effects, and at approximately 400 x lower dose-levels. Finally, microscopic nasal polypoid adenomas were induced in the rat when acetochlor was administered for two years at the maximum tolerated dose (MTD). The tumours were not life-threatening, they did not metastasize, and no DNA damage was induced in the nasal cells of rats maintained on a diet containing the MTD of acetochlor for either 1 or 18 weeks (comet assay). In order to probe the mechanism of action of these high dose toxicities a series of chemical and genetic toxicity studies was conducted on acetochlor and a range of structural analogues. These revealed the chloroacetyl substructure to be the clastogenic species in vitro. Although relatively inert, this substituent is preferentially reactive to sulphydryl groupings, most evidently, to glutathione (GSH). Similar chemical reactivity and clastogenicity in vitro was observed for two related chemicals bearing a chloroacetyl group, both of which have been defined as non-carcinogens in studies reported by the US. NTP. These collective observations indicate that the source of the clastogenicity of acetochlor in vitro is also the source of its rapid detoxification in the rat in vivo, via reaction with GSH.

Bond JA, Himmelstein MW, Seaton M, Boogaard P, Medinsky MA. **Metabolism of butadiene by mice, rats, and humans: a comparison of physiologically based toxicokinetic model predictions and experimental data**. Toxicology 1996;113(1-3):48-54.

BIOSIS COPYRIGHT: BIOL ABS. 1,3-Butadiene is a carcinogen in rats and mice, with mice being substantially more sensitive than rats. Our recent research is directed toward obtaining a better understanding of the cancer risk of butadiene in humans by evaluating species-dependent differences in the formation of the toxic metabolites epoxybutene and diepoxybutane. The recent data include in vitro studies on butadiene metabolism using tissues from humans, rats, and mice as well as experimental data and physiological model predictions for butadiene in blood and butadiene epoxides in blood, lung, and liver after exposure of rats and mice to inhaled butadiene. The findings suggest that humans would be more like rats and less like mice regarding the formation of butadiene epoxides. These research findings permit a reassessment of some default options that are used in carcinogen risk assessments. The research approach employed can be a useful strategy for developing mechanistic and toxicokinetic data to supplant default assumptions used in carcinogen risk assessments.

Clayson DB, Iverson F. **Cancer risk assessment at the crossroads: the need to turn to a biological approach**. Regul Toxicol Pharmacol 1996;24(1 Pt 1):45-59.
BIOSIS COPYRIGHT: BIOL ABS. Mathematically based carcinogen risk assessment is based on a number of prudent default assumptions which are becoming progressively less tenable as new scientific evidence is adduced. For example, the assumptions that all rodent carcinogens will be carcinogenic in humans and that there is no safe dose of any carcinogen may, in specific examples, be shown to be untrue. The mechanisms by which carcinogens exert their effects, especially the induction of DNA lesions, DNA repair of these lesions, and cell proliferation, are considered; it is suggested that with recently developed experimental techniques they might be employed to develop a more biologically based approach to risk assessment and might avoid at least, some of the pitfalls associated with the present mathematically based carcinogen risk assessment models. They might lead to an improved appreciation of the shape of the carcinogen dose-response curve, at least at medium to high exposure

levels.

Dosch J, Kaina B. **Induction of c-fos, c-jun, junB and junD mRNA and AP-1 by alkylating mutagens in cells deficient and proficient for the DNA repair protein O6-methylguanine-DNA methyltransferase (MGMT) and its relationship to cell death, mutation induction and chromosomal instability**. Oncogene 1996;13(9):1927-35.
An early and immediate response of cells upon irradiation with UV light and various other forms of genotoxic stress is the induction of the proto-oncogenes c-fos and c-jun. To address the questions of whether (a) methylating agents that are powerful carcinogens are effective in induction of fos and jun mRNAs, (b) induction is affected by the repair capacity of the cells, and (c) induction is accompanied by genotoxic effects, the levels of c-fos, c-jun, junB and junD mRNA were analysed in isogenic Chinese hamster cell lines deficient (phenotypically Mex-) and proficient (Mex+) for the DNA repair protein O6-methylguanine-DNA methyltransferase (MGMT) after treatment with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and methyl methanesulfonate (MMS). Both methylating agents were very effective in inducing fos and jun mRNAs, although they differ markedly in their potency to induce O6-methylguanine in DNA. Most responsive were c-fos and c-jun (up to 80-fold increases in mRNA level) whereas junB (up to ninefold) and junD (up to twofold) displayed an intermediate and weak response, respectively. No difference in the dose-dependence of induction of these mRNAs was observed between Mex- and Mex+ cells indicating that the critical genotoxic and mutagenic lesion induced by MNNG, i.e. O6-methylguanine, which is rapidly repaired by MGMT, does not act as a trigger for this response. Induction of fos and jun mRNAs by MNNG and MMS was accompanied by a dose-dependent increase in the activity of the transcription factor AP-1. To induce fos and jun mRNAs as well as AP-1, doses of MNNG were required which were more than 50-fold higher than those inducing gene mutations, recombination events (SCEs) and reproductive cell death, and fivefold higher than those inducing chromosomal aberrations in Mex cells. Therefore, the immediate induction of fos and jun mRNAs and AP-1 in Mex- cells upon their exposure to MNNG appears not to be essential for the generation of MNNG-induced mutagenic and genotoxic effects, which is possibly due to the high genotoxic potential of non-repaired O6-methylguanine. However, for MMS and UV light, which was included in this study for comparison, c-fos, c-jun, junB and junD mRNA as well as AP-1induction paralleled the dose-response for induction of cell killing effects, recombination and chromosomal breakage indicating that increased expression of Fos and Jun is related to the generation of MMS and UV-induced genetic changes. These data are in line with a model according to which the induced c-Fos and Jun proteins are involved in defense against UV radiation and other DNA damaging agents.

Dycaico MJ, Stuart GR, Tobal GM, De Boer JG, Glickman BW, Provost GS. **Species-specific differences in hepatic mutant frequency and mutational spectrum among lambda/lacI transgenic rats and mice following exposure to aflatoxin B1**. Carcinogenesis 1996;17(11):2347-56.
BIOSIS COPYRIGHT: BIOL ABS. In vivo mutations were studied in lambda/lacI (Big Blue) transgenic C57BL/6 mice and F344 rats following exposure to either AFB1 (aflatoxin B1) or DMSO vehicle. Fourteen days after exposure, livers were removed for DNA extraction and subsequent mutational analysis of the lacI gene. Mice injected with a single i.p. dose of AFB, at 2.5 mg/kg did not show a significant increase in liver mutant frequency relative to vehicle-treated controls. DNA sequence analysis of lacI mutations collected from the AFB1-treated mice showed a pattern of mutation similar to

that of the previously observed spontaneous mouse liver mutational spectrum. In contrast, rats subjected to one-tenth the mouse AFB1 dosage responded with an approximate 20-fold induction in liver mutant frequency over background. Sequencing of lacI mutations also revealed spectral differences between vehicle- and AFB1-treated rats. A large increase in G:C-T:A transversions was observed among lacI mutations isolated from the AFB1-treated rats. This work is among the first multi-species in vivo mutagenicity studies using transgenic rodents harboring the same shuttle vector. Such multi-species in vivo assays may prove to be valuable in the areas of mechanistic analysis and risk assessment.

Fahrig R. **Anti-recombinogenic and convertible co-mutagenic effects of (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU) and other 5-substituted pyrimidine nucleoside analogs in S. cerevisiae MP1**. Mutat Res 1996;372(1):133-9.
In experiments using yeast, without addition of an external metabolic activation system, (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU) was co-mutagenic and showed an insignificant anti-recombinogenic effect in combination with triethylene melamine (TEM). In the presence of activating S9-mix, the anti-recombinogenicity and co-mutagenicity could clearly be seen. At higher concentrations the co-mutagenic effect was converted into anti-mutagenicity. The other three 5-substituted pyrimidine nucleoside analogs were tested only in the presence of activating S9-mix and showed similar effects. As TEM is a direct alkylating agent that is inactivated by liver microsomes, the higher activity in presence of S9-mix can be interpreted as resulting from metabolic activation of the 5-substituted pyrimidine nucleoside analogs. In previous experiments using yeast bacteria, Drosophila or mice, tumor promoters were co-recombinogenic/anti-mutagenic, and co-carcinogens were co-mutagenic/anti-recombinogenic. Thus, there is not only an operational difference between tumor promoters and co-carcinogens but a real difference in respect to their genetic effectiveness. As up to now only co-carcinogens have shown co-mutagenic and anti-recombinogenic effects, it is perhaps possible that, within a certain concentration range, 5-substituted pyrimidine nucleoside analogs may have co-carcinogenic activity in carcinogenicity tests. At higher concentrations the co-carcinogenic effect may be converted into an anti-carcinogenic one.

Gray GM, Li P, Shlyakhter I, Wilson R. **An empirical examination of factors influencing prediction of carcinogenic hazard across species**. Regul Toxicol Pharmacol 1995;22(3):283-91.
This study was stimulated by a recent U.S. Environmental Protection Agency (EPA, 1994) statement in draft environmental carcinogen risk assessment guidelines: Several kinds of observations from animal studies can contribute to the judgment whether animal responses indicate a significant carcinogenic hazard to humans. We have investigated each of these kinds of observation using the cancer bioassay data system database. We obtained concordances from rat to mouse (and vice versa) for various subgroups of chemicals as follows: chemicals that induced tumors at multiple sites, chemicals that induce cancer in both sexes, chemicals that display reduced latency, and chemicals increasing the rates of rare tumors. The concordances are much higher for these chemical subgroups than the chemical groups that induce tumor at a single site, in only one sex, or without reduced latency, respectively. Thus, our findings support some of the EPA's suggested factors.

Guengerich FP, Kim MS, Muller M, Lowe LG. **Chemical mechanisms of formation of DNA-carcinogen adducts, elucidation of potential of adducts for mutagenicity, and mechanisms of polymerase fidelity and mutation in the presence of adducts**. Recent Results Cancer Res

1997;143:49-63.

Haas I, Koldovsky P, Ganzer U. **Exposure of organ cultures from human tracheal epithelium to chemical carcinogens and subsequent long-term co-cultivation with autologous isotopic fibroblasts**. Eur Arch Otorhinolaryngol 1996;253(7):405-10.
As a continuation of previous experiments introducing an extracorporeal model for transformation of human respiratory epithelium that might be able to mimic a spontaneously occurring malignant tumor, we prepared organ cultures from tracheal specimens and exposed them repeatedly to chemical carcinogens, using benzo(a)pyrene and methylnitronitrosoguanine for 6 weeks. We then tried to select possibly initiated cells by subsequent co-cultivation with autologous isotopic fibroblasts for 2 years. Nontreated controls were maintained from the same specimens and cultured in the same manner. By this technique we selected from specimen La24 three long-living cell lines with varying morphology and an antigenic pattern indicating dedifferentiation. The cells expressed simultaneously a panel of cytokeratins, vimentin and neuroectodermal antigens. Transplantation of these cell lines under the subrenal capsule of athymic mice resulted in tumorlike nodules of limited size. Success rate was dependent on time of previous in vitro culture and carcinogen treatment. None of the lines produced invasive or metastasizing tumors.

Hader C, Hadnagy W, Seemayer NH. **A rapid method for detection of nongenotoxic carcinogens of environmental pollutants using synchronized V79 cells and flow cytometry**. Toxicol Lett 1996; 88(1-3):99-108.
Synchronized V79 cells were treated before entering mitosis with known and suspicious mitotic arrestants and analyzed by flow cytometry and by light microscopy. Colcemid, nocodazole, vinblastine, diethylstilbestrol, triethyl lead and cadmium sulfate caused a dose dependent mitotic arrest of up to 80%, in comparison with 6% for the controls. Mixtures of polycyclic aromatic hydrocarbons and heterocyclic compounds induced a mitotic arrest of 50%-60%. Extracts of airborne particulates revealed a mitotic arrest of 10%-40%. In contrast, benzoquinone and hydroquinone led to a G2-block rather than to a mitotic arrest. Results of flow cytometry measurements correlated well with those obtained by light microscopy. Cell synchronization in combination with flow cytometry seems to be of considerable value as a rapid method for testing nongenotoxic agents with mitotic arresting activity.

Hartwig A, Schlepegrell R, Dally H, Hartmann M. **Interaction of carcinogenic metal compounds with deoxyribonucleic acid repair processes**. Ann Clin Lab Sci 1996;26(1):31-8.
The potentials of nickel(II) and cadmium(II) to interfere with the repair of different types of deoxyribonucleic acid (DNA) lesions was investigated. Concerning the nucleotide excision repair pathway, nickel(II) has been shown to reduce the incision and the ligation frequency after ultraviolet (UV)-irradiation. When applying a gel mobility shift assay and HeLa nuclear cell free extracts, nickel(II) diminishes the specific binding of a protein to UV-damaged DNA, suggesting that nickel(II) interferes with the DNA-protein interactions involved in the damage recognition after UV-irradiation. Similarly, the incision frequency is reduced in the presence of low concentrations of cadmium(II). Concerning the repair of oxidative DNA damage induced by visible light, non-cytotoxic concentrations of nickel(II) caused a complete repair inhibition of DNA base modifications like 7,8-dihydro-8-oxoguanine (8-hydroxyguanine) and of DNA strand breaks. Since the repair of DNA damage is essential for the

prevention of cancer, its inhibition may account for the carcinogenic action of the respective metal compounds.

Holzman P. **New criteria established for assessing human carcinogens [news]**. J Natl Cancer Inst 1996;88(23):1713-4.

Isfort RJ, Leboeuf RA. **Application of in vitro cell transformation assays to predict the carcinogenic potential of chemicals**. Mutat Res 1996;365(1-3):161-73.
Genotoxicity test batteries have become a standard fool for identifying chemicals that may have potential carcinogenic risk to humans. It is now apparent, however, that the use of genotoxicity batteries for assessing carcinogenic potential has limitations including an overall low specificity and a limited ability to detect carcinogens acting via 'nongenotoxic' mechanisms. In vitro cell transformation models, because they measure a chemical's ability to induce preneoplastic or neoplastic endpoints regardless of mechanism, may fulfil the current need for an in vitro biologically relevant model with increased predictiveness for determining carcinogenic potential. This review will focus on data demonstrating the similarities of chemically induced cell transformation in vitro to carcinogenesis in vivo. Furthermore, a growing database demonstrating a high overall correlation between cell transformation results with those of the rodent bioassay will also be discussed. Finally, the inclusion of cell transformation approaches for assessing the carcinogenic potential of chemicals relative to currently used genotoxicity batteries will be presented.

Kato T, Harashima T, Moriya N, Kikugawa K, Hiramoto K. **Formation of the mutagenic/carcinogenic imidazoquinoxaline-type heterocyclic amines through the unstable free radical Maillard intermediates and its inhibition by phenolic antioxidants**. Carcinogenesis 1996;17(11):2469-76.
Generation of the imidazoquinoxaline-type heterocyclic amines in the heated model system composed of glucose/glycine/creatinine in aqueous diethylene glycol was effectively prevented by phenolic antioxidants, butylated hydroxyanisole (BHA), propyl gallate (PG), sesamol, esculetin and epigallocatechin gallate (EGCG) in a dose-dependent manner. Generation of the mutagens in heated-and-dried bonito meat was effectively prevented on pretreatment with EGCG or green tea extract. Electron spin resonance (ESR) studies showed that the heated model mixture of glucose/glycine generated the unstable pyrazine cation radical, and its formation was inhibited by BHA, sesamol and EGCG. ESR-spin trapping studies using 5,5-dimethyl-1-pyrroline N-oxide (DMPO) and N-tert-butyl-alpha-phenylnitrone (PBN) showed that the heated model mixture of glucose/glycine or glucose/glycine/creatinine generated unstable carbon-centred radical(s), and their formation was effectively inhibited by BHA, sesamol and EGCG. It is likely that the unstable free radical Maillard intermediates played an important role in the formation of the imidazoquinoxaline-type heterocyclic amines, and the phenolic antioxidants effectively scavenged the radical species to prevent the mutagen formation.

Kensler TW, Groopman JD, Wogan GN. **Use of carcinogen-DNA and carcinogen-protein adduct biomarkers for cohort selection and as modifiable end points in chemoprevention trials**. IARC Sci Publ(139) 1996:237-48.
Clinical cancer prevention studies that use disease as an end point are of necessity large, lengthy and extremely costly. Development of the field of cancer chemoprevention is being accelerated by the

application of intermediate markers to preclinical and clinical studies. One class of potentially useful biomarkers comes from studies of cancer risks derived from exposure to environmental carcinogens of both endogenous and exogenous origins. Sensitive and specific analytical methods have been developed for detecting and quantifying levels of covalent adducts of several important classes of carcinogens with cellular DNA and blood proteins at ambient levels of exposure. Such biomarkers can be applied to the preselection of exposed individuals for study cohorts, thereby reducing study-size requirements. Levels of these carcinogen-DNA and carcinogen-protein adducts can be modulated by some classes of chemopreventive agents. In this regard, these markers can also be used to rapidly assess the efficacy of preventive interventions such as exposure abatement and chemoprevention. However, the successful application of these biomarkers to prevention trials will be dependent upon prior determination of the associative or causal role of the marker to the carcinogenic process, establishment of the relationship between dose and response, and appreciation of the kinetics of adduct formation and removal.

Kerckaert GA, Leboeuf RA, Isfort RJ. **Use of the Syrian hamster embryo cell transformation assay for determining the carcinogenic potential of heavy metal compounds**. Fundam Appl Toxicol 1996;34(1):67-72.
BIOSIS COPYRIGHT: BIOL ABS. Cobalt sulfate hydrate, gallium arsenide, molybdenum trioxide, vanadium pentoxide, and nickel sulfate heptahydrate were tested in the Syrian hamster embryo (SHE) assay in order to increase the SHE assay database for heavy metals. All five compounds produced significant morphological transformation at one or more doses in a dose-responsive manner. Cobalt sulfate hydrate, gallium arsenide, molybdenum trioxide, and nickel (II) sulfate heptahydrate were all positive with a 24-hr exposure, suggesting direct DNA perturbation. Vanadium pentoxide was negative with a 24 hr exposure, but positive with a 7-day exposure. This pattern of response (24-hr SHE negative/7-day SHE positive) has been seen with other chemicals which have tumor promotion-like characteristics. Since the inception of the use of the SHE cell transformation assay for detecting the neoplastic transformation potential of chemicals, over 42 heavy metal compounds have been tested in this assay. Based on the 24 metal compounds which have been tested in the SHE, Salmonella, and some type of rodent bioassay, the SHE assay is 92% concordant with rodent bioassay carcinogenicity results, including a sensitivity of 95% (21/22) and a specificity of 50% (1/2). At this time, the measure of SHE assay specificity for rodent carcinogenicity of metals is limited by the paucity of metal compounds which are rodent noncarcinogens. The Salmonella assay results are only 33% concordant with the rodent bioassay for these same chemicals. This relatively high concordance between the SHE assay and the rodent bioassay carcinogenicity results demonstrates the utility of the SHE assay for determining the carcinogenic potential of heavy metal compounds in rodent cancer bioassays.

Kuchenmeister F, Wang M, Klein RG, Schmezer P. **Transport of reactive metabolites of procarcinogens between different liver cell types, as demonstrated by the single cell microgel electrophoresis assay**. Toxicol Lett 1996;88(1-3):29-34.
Procarcinogens have to be activated by specific cytochromes before showing adverse effects. Freshly isolated hepatocytes (parenchymal liver cells, PC) are characterized by a high content of such xenobiotic enzymes and are widely used to investigate chemically induced DNA damage. But in many cases liver tumors caused by indirect acting carcinogens can also originate from non-parenchymal liver cells (NPC). We used freshly isolated rat PC and NPC to demonstrate that only PC have activation capacity

when treated in vitro with different genotoxic procarcinogens (N-nitrosodimethylamine, NDMA; vinyl chloride, VC). The alkaline single cell microgel electrophoresis assay was applied to measure the genotoxic activity of the activated compounds. In order to test the hypothesis that reactive metabolites can be transported from PC to NPC, we performed additional in vivo studies as well as studies in which PC were incubated together with NPC, only separated by a dialysis tube (in vitro coincubation). The results indicate that reactive metabolites of both NDMA and VC are stable enough to be transported intercellularly from PC to NPC.

Kurihara T, Motohashi N, Pang GL, Higano M, Kiguchi K, Molnar J. **Correlations between topological resonance energy of methyl-substituted benz[c]acridines, benzo[a]phenothiazines and chrysenes, and their carcinogenic or antitumor activities**. Anticancer Res 1996;16(5a):2757-65.
In order to clarify the effects of methyl substitution on the carcinogenic activity, each resonance energy (RE) of benz[c]acridines, benzo[a]phenothiazines, chrysene, and their methyl derivatives was calculated by Aihara's TRE theory. Some correlations seem to exist between the values of resonance energy per pi-electron for the cationic species-with the lack of the atom having the highest approximate superdelocalizability (Sr'(E)) from their parents skeleton-and carcinogenic activity.

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

Lutz W, Krajewska B. **[Oncoproteins and anti-oncoproteins in blood serum as biomarkers of early health effects induced by occupational and environmental carcinogens]**. Med Pr 1996;47(5):511-8. (Pol)
Over a whole life-span a genetic machinery of human cells is exposed to carcinogenic effect of various

chemical, physical and biological factors leading to cellular changes in the genome like point mutation and translocation or amplification of genes. Together with growing an ageing of the human organism, the number and heterogeneity of mutated cells increase. Thus, the presence or absence of the neoplastic process may depend on the length of life, efficiency of the immunological system, cumulation of adverse affects of environmental factors or inherited susceptibility to a disease. There is no doubt that neoplasms which develop due to inheritance of different genetic defects or due to direct environmental effect progress in line with similar mutations, resulting finally in the genome destabilisation and disturbances in the balance between proliferation and differentiation processes. The knowledge as to how far environmental carcinogens affect cellular genome is still limited. Nevertheless, data collected to date help to understand the mechanisms by which environmental carcinogens may initiate the process of transforming normal cells into neoplastic ones. It is most likely that activation of some oncoproteins and deactivation of some suppressive genes and related qualitative and quantitative changes in oncoproteins and anti-oncoproteins participating in the growth control and cellular division, are the essential mechanisms which are involved in this process. The discovery that activation of oncoproteins and related emergence of oncoprotein in cells, increased in the number of changed, can be monitored in easily available biological fluids, such as blood or urine, has become of great importance for occupational medicine and environmental health. That has provided new diagnostic measures for evaluating carcinogenic effects of the working and living environments. The fact, that the emergence of oncoproteins in biological fluids may precede by many months or even years clinical manifestations of neoplastic disease should greatly contribute to undertaking more effective preventive measures.

Malins DC, Polissar NL, Garner MM, Gunselman SJ. **Mutagenic DNA base modifications are correlated with lesions in nonneoplastic hepatic tissue of the English sole carcinogenesis model**. Cancer Res 1996;56(24):5563-5.
Hydroxyl radical-induced mutagenic base modifications have been linked to neoplasia in a number of biological systems, including English sole from chemically contaminated urban environments. However, virtually no information exists on the relationship between the mutagenic base modifications and preneoplastic and other lesions found in tumor-free tissues prone to cancer. We studied six hepatic lesions in immature, neoplasm-free English sole exposed to an urban and reference environment and established correlations between the lesion incidence and concentrations of the mutagenic base modifications 8-hydroxyguanine and 8-hydroxyadenine. The lesions were putatively preneoplastic basophilic foci, hepatocellular karyomegaly, megalocytic hepatosis, hepatocellular vacuolar change, hyalin droplet formation, and apoptosis. With the exception of hepatocellular vacuolar change, significant positive correlations were found between the lesions and the mutagenic base modifications. The hydroxyl radical may be a common etiological factor in the formation of the base modifications and hepatic lesions.

Marchant CA. **Prediction of rodent carcinogenicity using the DEREK system for 30 chemicals currently being tested by the National Toxicology Program**. Environ Health Perspect Suppl 1996;104 (5):1065-73.
CBAC COPYRIGHT: CHEM ABS DEREK is a knowledge-based expert system for the qual. prediction of toxicity. The DEREK system has been used to predict the carcinogenicity in rodents of the 30 chems. in the second National Toxicol. Program (NTP) carcinogenicity prediction exercise. Seven of the chems.

were predicted to be carcinogens. For 23 chems., there was no evidence in the DEREK knowledge base to suggest carcinogenic activity. Supplementary data from a variety of sources have been evaluated by human experts to assess confidence in each DEREK prediction. These sources included std. toxicol. ref. texts, genotoxicity and subchronic toxicity assay results for each chem., as well as Salmonella mutagenicity and carcinogenicity.

Mattagajasingh SN, Misra HP. **Mechanisms of the carcinogenic chromium(VI)-induced DNA-protein cross-linking and their characterization in cultured intact human cells**. J Biol Chem 1996;271(52):33550-60.
DNA-protein complexes (DPCs) were induced in human leukemic T-lymphocyte MOLT4 cells by treatment with potassium chromate. DPCs were isolated by ultracentrifugal sedimentation in the presence of 2% SDS and 5 M urea. The complexes were analyzed by two-dimensional SDS-polyacrylamide gel electrophoresis. Three acidic proteins of 74, 44, and 42 kDa and a basic protein of 51 kDa were primarily complexed to DNA following 25 &mgr;M chromate treatment. Higher concentrations of chromate cross-linked many other proteins to DNA. Amino acid sequencing and immunoblotting studies indicated that the acidic 44-kDa protein could be nuclear beta-actin. Lectin and aminoglycoside nucleotidyltransferase were also found to cross-link with DNA by chromate treatment. The composition and stability of the DPCs were studied using nucleases, proteinase K, and disruptive chemicals. Pretreatment of cells with antioxidants inhibited the formation of DPCs, measured as K+-SDS precipitable DPCs, indicating the involvement of oxidative mechanisms. Because chromate causes certain nuclear beta-actin. Lectin and aminoglycoside nucleotidyltransferase were also found to cross-link with DNA by chromate treatment. The composition and stability of the DPCs were studied using nucleases, proteinase K, and disruptive chemicals. Pretreatment of cells with antioxidants inhibited the formation of DPCs, measured as K+-SDS precipitable DPCs, indicating the involvement of oxidative mechanisms. Because chromate causes certain nuclear proteins to form complexes with DNA and the complexes are resistant to treatments such as 2% SDS and 5 M urea, but disruptable under gel electrophoretic conditions, chromium could be used as a cross-linking agent for the identification of other proteins, such as transcription factors, that transiently interact with DNA.

McDonald AL, Fielder RJ, Diggle GE, Tennant DR, Fisher CE. **Carcinogens in food: priorities for regulatory action**. Hum Exp Toxicol 1996;15(9):739-46.
A pragmatic possible approach to the prioritization of chemical carcinogens occurring as food contaminants is described, based on the carcinogenic risk to the population. This should be of value in ensuring that resources for assessment and management of carcinogens in food are directed to the most important areas with regard to carcinogenic risk to the population. Key components of this approach are an assessment of the carcinogenic hazard to humans combined with estimations of intakes per person and of the proportion of the population exposed. These are used to derive an index referred to as the Population Carcinogenic Index. Concerning the hazard assessment expert judgement is used to place the chemical in one of five categories. The highest category is for chemical carcinogens that are believed to act by a genotoxic mechanism. It is recognised that such compounds may vary enormously with respect to their potency and various approaches to ranking carcinogens on the basis of potency are reviewed. The approach adopted is to subdivide the genotoxic carcinogens category into high, medium and low potency based on the TD50 value. Methods of estimating intakes and exposed populations are

considered and an approach which groups these into broad categories is developed. The hazard and exposure assessments are then combined to derive the Population Carcinogenicity Index.

Morimoto K, Kimura M, Murata T, Imai Y, Ookami N, Igarashi T, Kanoh N, Kaminuma T, Hayashi Y. **[An in vivo DNA adduct database for carcinogens: O6-alkylguanine, O4-alkylthymine and 8-hydroxyguanine]**. Eisei Shikenjo Hokoku 1994;(112):17-26. (Jpn)
Many carcinogens react with DNA and form critical DNA adducts, such as O6-alkylguanine (O6-AG), O4-alkylthymine (O4-AT), and 8-hydroxyguanine (8-OHG). This study provides a database that can be used for molecular dosimetry of these DNA adducts. A literature survey on DNA binding in vivo was done by the Dialog search from the MEDLINE database. We propose a Critical Covalent Binding Index (CCBI) for the assessment of in vivo DNA binding level (expressed as micro mol chemical bound per mol G or T/mmol chemical administered per kg body weight). The number of records and compounds in parenthesis of O6-AG, O4-AT, and 8-OHG were 245(13), 54(4), 79(15), respectively. Since the CCBI values for N-nitrosamine in target organ were higher than for non-target organ, they may provide a useful index for estimation of target organ site and carcinogenic potency. As a case example, CCBI values for O4-AT from animal data were applied for diethylnitrosamine human exposure estimation by diethylnitrosamine.

Nersesian AK. **[The micronucleus test in human exfoliative cells as a method for studying the action of mutagens/carcinogens]**. Tsitol Genet 1996;30(5):91-6. (Rus)
The data concerning the application of micronucleus assay in human exfoliated cells have been analyzed in the review. These data have shown that the study of micronuclei in human exfoliated cells is very promising, sensitive and rapid method for detection of action of mutagens/carcinogens as well as antimutagens/anticarcinogens on human organism in vivo.

Pool-Zobel BL, Neudecker C, Domizlaff I, Ji S, Schillinger U, Rumney C, Moretti M, Vilarini I, Scassellati-Sforzolini R, Rowland I. **Lactobacillus- and bifidobacterium-mediated antigenotoxicity in the colon of rats**. Nutr Cancer 1996;26(3):365-80.
Lactic acid bacteria (LAB) are proposed to have several beneficial effects, including the inactivation of carcinogens. We have studied the potential of Lactobacillus acidophilus (from a commercially available yogurt), Lactobacillus gasseri (P79), Lactobacillus confusus (DSM20196), Streptococcus thermophilus (NCIM 50083), Bifidobacterium breve and Bifidobacterium longum (from human infant stool) to prevent the induction of DNA damage by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG, 7.5 mg/kg body wt) in colon cells of the rat. Using the new technique of single cell microgel electrophoresis, all investigated strains were antigenotoxic toward MNNG after a single dose of 10(10) viable cells/kg body wt p.o. eight hours before the carcinogen. One-half and one-tenth of this initial dose resulted in a loss of protective activity. High doses of heat-treated L. acidophilus strains were also not antigenotoxic. One mechanism of the preventive effect could be that bacterial metabolites or components are responsible. Accordingly, selected examples were investigated in vitro in colon cells of the rat. Metabolically active L. acidophilus cells, as well as an acetone extract of the culture, prevented MNNG-induced DNA damage. Different cell fractions from L. acidophilus (cytoplasm, cell wall skeleton, cell wall) were devoid of antigenotoxic activity, whereas the peptidoglycan fraction and whole freeze-dried cells were antigenotoxic. As a second carcinogen, 1,2-dimethylhydrazine (DMH) was used. A dose- and time-response study was first performed to assess the effects of DMH in several segments of the

gastrointestinal (GI) tract. Exposure for 16 hours to 15 or 25 mg DMH/kg body wt p.o. induced DNA damage in cells of the distal colon of rats, whereas no cytotoxicity was seen. Pretreatment orally with LAB on four consecutive mornings before DMH gavage (8 hours after the last LAB application) revealed that L. acidophilus, L. confusus, L. gasseri, B. longum, and B. breve inhibited the genotoxic effect of DMH. One of four S. thermophilus and one of three Lactobacillus delbrueckeii ssp. bulgaricus strains were also protective. Heat-treated L. acidophilus did not inhibit DMH-induced genotoxicity. A few aliquots of the colon cells were processed immunohisto-chemically for the presence of the proliferation cell nuclear antigen (PCNA). DMH treatment did not increase PCNA, nor was there any modulation by LAB. The effect of L. acidophilus on foreign compound-metabolizing enzymes (Phase I and Phase II) in liver and colon cells of rats revealed only one parameter to be modulated, namely, a two- to three-fold increase in the levels of NADPH-cytochrome P-450 reductase. The meaning of this finding, in terms of possible chemoprevention by LAB, remains unclear. In conclusion, our studies show that most, but not all, LAB tested could strongly inhibit genotoxicity in the GI tract of the rat and that viable LAB organisms are required for the protective effect in vivo. The comet assay technique is a powerful tool to elucidate such in vivo antigenotoxic activities in tumor target tissues.

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

Rhomberg L. **Risk assessment and the use of information on underlying biologic mechanisms: a perspective**. Mutat Res 1996;365(1-3):175-89.
Recent years have seen the rapid expansion of scientific understanding of the underlying biologic bases of toxic reactions to chemicals. Use of this information in health risk assessment is expanding, but it has yet to reach its full potential. This article considers what has successfully been done, what approaches are now being developed, and what impediments and difficulties have been encountered in attempts to bring case-specific, mechanistic toxicological information to bear on risk estimation. In hazard identification, mechanistic information can help explain the bearing of various empirical experimental results for inferring human hazard, can increase the sensitivity of detection, and can be considered in

attempts to replace 2-year animal bioassays with hazard identification methods that rest on identifying key biological properties underlying carcinogenicity rather than relying only on the experimental observation of tumors. In carcinogen potency estimation, mechanistic information can potentially extend relevant observation to lower dose levels, provide the basis for choosing among empirically based dose-response models, lead to potency estimates through relationships with quantitative measures of short-term test outcomes, and can be considered as a basis for providing direct observation of the biological parameters in biologically based dose-response modeling.

Ruder AM. **Epidemiology of occupational carcinogens and mutagens**. Occup Med 1996;11(3):487-512.
The author outlines the methods by which the International Agency for Research on Cancer evaluates study design and results when it reviews epidemiologic studies to determine carcinogenicity and mutagenicity. The chapter concludes with an extensive series of tables summarizing (1) the tests relevant to mutagenicity and (2) the IARC rating system for carcinogens, categorizing industrial and agricultural chemicals according to evidence of mutagenicity.

Saikawa A, Nomura T, Yamashita F, Takakura Y, Sezaki H, Hashida M. **Pharmacokinetic analysis of drug disposition after intratumoral injection in a tissue-isolated tumor perfusion system**. Pharm Res 1996;13(10)1438-44.
CBAC COPYRIGHT: CHEM ABS The purpose of this study was to establish an exptl. system for evaluation of the intratumoral behavior of drugs after intratumoral injection using perfused tissue-isolated tumor prepns. of Walker 256 carcinoma (3.46-9.73 g). We quantified the recovery of Phenol Red (model drug) in the tumor, leakage from the tumor surface and the venous outflow after intratumoral injection using perfused tissue-isolated tumors, and analyzed venous appearance curves based on a pharmacokinetic model in which the tumor tissue was assumed to be divided into two compartments, i.e., well- and poorly-perfused regions. In small tumors (Type 1, 5.42 g), the drug appeared immediately in the venous outflow, and the amt. remaining in the tumor tissue at 2 h after injection was small. In contrast, the venous appearance rate reached a significantly lower peak a few minutes after injection, and a large amt. of injected drug remained in some large tumors (Type 2, 8.17 g). Pharmacokinetic anal. revealed that there was a correlation between tumor wt. and the rate consts. of transfer from the poorly-perfused region to the well-perfused region, and between the rate consts. of transfer from the well-perfused region t the venous outflow and dosing ratios into the well-perfused region. An exptl. system and anal. method were established for the evaluation of the intratumoral behavior of drugs after intratumoral injection using a tissue-isolated tumor perfusion system. This exptl. system will be useful in analyzing the antitumor drug disposition after intratumoral injection.

Schartl A, Pagany M, Engler M, Schartl M. **Analysis of genetic factors and molecular mechanisms in the development of hereditary and carcinogen-induced tumors of Xiphophorus**. Recent Results Cancer Res 1997;143:225-35.

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 results from assays that describe biological effects of polycyclic aromatic hydrocarbons (PAH) were explored using multivariate methods. Based on the availability of data, 29 PAH were included in the study. Five variables described the carcinogenic potency in rodents of the PAH. Biological effects were assayed using 14 variables. These included bacterial mutagenicity, enhancement and inhibition of bacterial mutagenicity, Ah receptor (AhR) affinity, and induction of enzymes that bioactivate many PAH to proximal bacterial mutagens. A principal components analysis (PCA) showed that the highest correlations with the cancer data were observed for variables describing AhR affinity, whereas bacterial mutagenicity data were poorly correlated with cancer data. When a partial least squares (PLS) regression analysis was applied, only one bacterial mutagenicity variable, but all AhR affinity variables were statistically relevant to describe carcinogenic potency. The latter variables were also correlated with the inhibition of bacterial mutagenicity of benzo[a]pyrene. It was concluded that structural requirements for AhR affinity are the same as those required for metabolism by enzymes that bioactivate benzo(a)pyrene. Negative correlations between mutagenicity and induction of enzymes were observed. The roles of cancer initiation and cancer promotion are discussed regarding the biological properties studied. It is proposed that bacterial mutagenicity reflects the cancer initiation potency, whereas the AhR affinity reflects the promotive effect of some PAH at the high doses applied in rodent carcinogenicity tests. It is thus indicated that initiation and promotion are provoked by different chemical species: reactive metabolites and the parent hydrocarbons, respectively. At doses reflecting a normal human exposure situation the effects of initiation may be more important in the course of chemical carcinogenesis. The mechanisms of cancer initiation and cancer promotion should therefore be studied in more detail for reliable quantitative risk assessments.

Stopper H, Eckert I, Wagener P, Schulz WA. **Formation of micronuclei and inhibition of topoisomerase II in the comet assay in mammalian cells with altered DNA methylation**. Recent Results Cancer Res 1997;143:183-93.

Tamir S, Tannenbaum SR. **The role of nitric oxide (NO) in the carcinogenic process**. Biochim Biophys Acta 1996;1288(2):F31-F36.
BIOSIS COPYRIGHT: BIOL ABS. The inflammatory process has long been known to be a risk factor for human cancers, particularly of the lung, bladder, colon, stomach, and female breast. Earlier hypothesis cited production of oxygen radicals, release of cytokines, and synthesis of prostaglandins and leukotrienes as biochemical modulators of the carcinogenic process. The discovery of NO as a product of cells in the immune system has implicated this chemical in the mechanism of carcinogenesis, particularly when NO is overproduced over a long period of time. After briefly reviewing the important chemical reactions of NO under physiological conditions, we examine how the chemistry of its key reactants toward biologically important molecules relate to DNA damage and cytotoxicity. In these two processes, NO may play an important role in currently accepted models of multistage carcinogenesis.

Tennant RW, Spalding J. **Predictions for the outcome of rodent carcinogenicity bioassays: Identification of trans-species carcinogens and noncarcinogens**. Environ Health Perspect 1996;104 (Suppl 5):1095-100.
BIOSIS COPYRIGHT: BIOL ABS. Thirty chemicals or substances currently undergoing long-term carcinogenicity bioassays in rodents have been used in a project to further evaluate methods and

information that may have the capability of predicting potential carcinogens. In our predictions the principal information used includes structural alerts and in vitro test results for Salmonella mutagenicity, relative subchronic toxicity, and the sites and types of pathology found in subchronic (90-day) studies. This group of chemicals differs significantly from those used previously to evaluate predictive methods in that 23 of 30 are defined as nonmutagenic by conventional criteria. The goal of this predictive effort is to identify categorically the chemicals that have the capacity to induce cancers in both rats and mice (trans-species carcinogens) and those that are not carcinogenic in either rats or mice. Chemicals that show properties that may be associated with tumor induction in either species, i.e., species-specific cancers, are categorized as being of uncertain predictability. This category includes chemicals believed to have limited carcinogenic potential that is manifested principally as a consequence of the genetic background of the test strain of inbred rodent.

Tennant RW, Spalding J, French JE. **Evaluation of transgenic mouse bioassays for identifying carcinogens and noncarcinogens**. Mutat Res 1996;365(1-3):119-27.
Data supporting the use of transgenic lines to identify carcinogens and noncarcinogens are thus far based on a limited number of chemicals for which there are also long-term bioassay results in rats and/or mice. Six chemicals have been tested in the heterozygous p53-deficient mice and 13 in the Tg.AC line. The results show that the p53def responds rapidly to mutagenic carcinogens and the Tg.AC responds rapidly to both mutagenic and nonmutagenic carcinogens. Neither transgenic line responded to the noncarcinogens that were tested. The p53def line failed to respond to two nonmutagenic carcinogens (N-methyloacrylamide and reserpine), the Tg.AC line failed to respond to ethyl acrylate, a nonmutagenic chemical that induced tumors of the forestomach when administered by gavage, and to triethanolamine that caused an increase in hepatocellular tumors in B6C3F1 mice via skin painting. Both of the latter chemicals are examples of highly specific responses related to either route of administration or to strain susceptibility. Further efforts to evaluate the range of chemicals to which these transgenic lines respond are currently in progress.

Watanabe M. **[Genetic and phenotypic polymorphisms in carcinogen-metabolizing enzymes and cancer susceptibility]**. Nippon Rinsho 1996;54(8):2261-75. (Jpn)
Most of chemical carcinogens require metabolic activation before they interact with cellular macromolecules and can cause cancer initiation. Many of cytochrome P-450 (CYP) mediating oxidative enzymes and conjugation enzymes, cloned and characterized in humans, show genetic and phenotypic polymorphisms and have been suggested to contribute to individual cancer susceptibility as genetic modifiers of cancer risk. Altered phenotypes and genotypes in CYP1A1, CYP2D6 and CYP2E1 and in defective glutathione S-transferase (GST) and N-acetyltransferase enzymes have been associated with an increased risk of developing lung and other cancers. The risk to lung cancer is dramatically increased in the population carried simultaneously high-risk genotypes in CYP1A1 and GSTM1. There are, however, several studies in each category in which no association have been found.

Wilkinson CF, Killeen JC. **A mechanistic interpretation of the oncogenicity of chlorothalonil in rodents and an assessment of human relevance**. Regul Toxicol Pharmacol 1996;24(1 Pt 1):69-84.
Chronic dietary treatment of rodents with the fungicide chlorothalonil causes an increased incidence of papillomas and carcinomas of the forestomach squamous epithelium (rats and mice, both sexes) and adenomas and carcinomas of the renal proximal tubule epithelium (rats, both sexes; mice, males only);

the product elicits no tumorigenic response in dogs. As a result, chlorothalonil is classified by EPA as a Group B2 probable human carcinogen. However, chlorothalonil is not genotoxic and there is strong evidence that both the forestomach and renal tumors observed in rodents result from cytotoxicity followed by compensatory cell proliferation and hyperplasia. In the case of the forestomach, cytotoxicity results from sustained irritation of the squamous epithelium by chlorothalonil leading to inflammation, ulceration, and restorative hyperplasia. Cytotoxicity in the renal tubular epithelium is associated with formation of di- and trithiols that arise through the action of renal beta-lyase on cysteine S-conjugates derived from the corresponding glutathione conjugates of chlorothalonil. Renal cytotoxicity and cell necrosis in rodents result from the ability of the di- and trithiols to inhibit kidney mitochondrial respiration and disrupt cellular integrity. There is strong evidence that this mechanism is not operative in other species such as dogs and monkeys. The progression from cytotoxicity to hyperplasia to neoplasia is becoming increasingly well-recognized as a threshold-based mechanism of carcinogenesis. Unless exposure is excessively prolonged or intense, the cytotoxic effects will be fully reversible. Furthermore, the effects observed in rodents are not appropriate for evaluating the potential human cancer risk from chlorothalonil. Humans do not possess an organ equivalent to the rodent forestomach and the rat is a poor model for evaluating potential human risk for the renal tumorigenicity of chlorothalonil. Humans are likely to be very much less sensitive than rats to the nephrotoxic effects of chlorothalonil. In view of the fact that the tumorigenic effects of chlorothalonil are mediated through a well-understood, nongenotoxic, threshold-based mechanism of little or no relevance to humans, chlorothalonil should be a prime candidate for re-review under EPA's new risk assessment guidelines. Expert committees in both Europe and Canada have concluded that human risks to chlorothalonil should be evaluated by means of the.

Wiltse J, Dellarco VL. U.S. **Environmental Protection Agency guidelines for carcinogen risk assessment: past and future**. Mutat Res 1996;365(1-3):3-15.
The U.S. Environmental Protection Agency (USEPA) recently proposed new guidelines to update and replace the 1986 USEPA Guidelines for Carcinogen Risk Assessment. Today, there is a better understanding of the variety of modes by which carcinogens can operate that did not exist when the 1986 USEPA guidelines were published. Many laboratories are adding new test protocols in their programs directed at questions concerning the mechanisms of action of carcinogens. In response to the evolving science of carcinogenesis, the new guidelines provide an analytical framework for incorporating all relevant biological information and recognizing a variety of situations regarding cancer risk. In addition, the guidelines are flexible enough to allow consideration of future scientific advances.

Yamamoto S, Mitsumori K, Kodama Y, Matsunuma N, Manabe S, Okamiya H, Suzuki H, Fukuda T, Sakamaki Y, Sunaga M, et al. **Rapid induction of more malignant tumors by various genotoxic carcinogens in transgenic mice harboring a human prototype c-Ha-ras gene than in control non-transgenic mice**. Carcinogenesis 1996;17(11):2455-61.
In this study, we investigated the carcinogenic response of transgenic mice carrying the human prototype c-Ha-ras gene, namely Tg rasH2/CB6F1 mice, to various genotoxic carcinogens and compared it with that of control non-transgenic CB6F1 mice (non-Tg mice). The present studies were conducted as the first step in the evaluation of the Tg rasH2/CB6F1 mouse as a model for the rapid carcinogenicity testing system. Short-term (< or = 6 months) rapid carcinogenicity tests of various genotoxic carcinogens, 4-

nitroquinoline-1-oxide, cyclophosphamide, N,N-diethylnitrosamine, N-methyl-N-nitrosourea, N-methyl-N'-nitro-N-nitrosoguanidine and methylazoxymethanol, revealed that Tg rasH2/CB6F1 mice are more susceptible to these genotoxic carcinogens than control non-Tg mice. Tg rasH2/CB6F1 mice developed tumors more rapidly compared with non-Tg mice. Malignant tumors were observed only in the carcinogen-treated Tg rasH2/CB6F1 mice, but not in non-Tg mice treated with the same carcinogens. Each carcinogen induced tumors in corresponding target tissues of the Tg rasH2/CB6F1 mice. Only a very few lung adenomas but no other tumors were seen as spontaneous tumors during the 6 months of carcinogenicity tests. These results demonstrate that more rapid onset and higher incidence of more malignant tumors can be expected with high probability after treatment with various genotoxic carcinogens in the Tg rasH2/CB6F1 mice than in control non-Tg mice. The Tg rasH2/CB6F1 mouse seems to be a promising candidate as an animal model for the development of a rapid carcinogenicity testing system.

Yamasaki H. **Role of disrupted gap junctional intercellular communication in detection and characterization of carcinogens**. Mutat Res 1996;365(1-3):91-105.
BIOSIS COPYRIGHT: BIOL ABS. Results from short-term tests for carcinogens and our advanced knowledge on cellular and molecular mechanisms of carcinogenesis strongly suggest that carcinogens do not induce genetic changes necessarily by directly interacting with DNA. Therefore, it is not surprising to see that many carcinogens are not detectable by available genetic toxicology tests. Thus, it has become necessary to study nongenotoxic mechanisms of carcinogenesis and to provide methods to predict those carcinogens which escape from conventional mutation tests. One possible nongenotoxic mechanism of carcinogenesis which is supported by abundant experimental evidence is inhibition of gap junctional intercellular communication. Many, but not all, tumor-promoting agents have been shown to inhibit the communication of cultured cells as well as in vivo. Molecular mechanisms of gap junctional intercellular communication control revealed that connexin (gap junction) genes form a family of tumor suppressor genes. Control mechanisms of expression as well as function of connexins are vulnerable to various carcinogenic insults, notably to nongenetoxic carcinogens. Thus, studies on the role of connexins in cell growth and carcinogenesis may prove to be useful for establishing a mechanism-based test to detect certain types of nongenotoxic carcinogens.

Yoshikawa K. **Anomalous nonidentity between Salmonella genotoxicants and rodent carcinogens: nongenotoxic carcinogens and genotoxic noncarcinogens**. Environ Health Perspect 1996;104(1):40-6.
According to current data, the capacity to cause nonprogrammed or unscheduled cell proliferation in target tissues, a common characteristic of chemical carcinogens, may play a more important role in the development of tumors than does genotoxicity. This paper provides strong support for the validity of this conclusion. Ames-negative nongenotoxicants may be considered to be carcinogenic primarily because of their ability to induce cell proliferation in animal tissues and organs. In addition, such nongenotoxic carcinogens may also provide latent and modest DNA (equivocal) modifications that never lead to Ames-positive events. Conversely, noncarcinogenesis by Ames-positive agents is likely to be linked to a lack of stimulation of cell division. Nongenotoxic and genotoxic carcinogens rely on both cell proliferation and equivocal DNA modification for their full carcinogenicity. Such equivocal DNA modifications do not appear to be formed by tumor promoters. The role of cell proliferation may provide a favorable milieu for the occurrence of genetic instability, give rise to selective apoptosis-resistant abnormal cells,

and then affect clonal expansion of these cells. Therefore, understanding the influence of nongenotoxic and genotoxic carcinogens on cell proliferation capability is a key point in determining the mechanisms of chemical carcinogenesis. Considering the contradictory and common features of genotoxicants and carcinogens, early detection of nonprogrammed cell proliferation is the most effective approach to predict human and rodent carcinogenicity.

Zhang XB, Felton JS, Tucker JD, Urlando C, Heddle JA. **Intestinal mutagenicity of two carcinogenic food mutagens in transgenic mice: 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine and amino (alpha)carboline**. Carcinogenesis 1996;17(10):2259-65.
The heterocyclic amines produced during the cooking of meat, including amino(alpha)carboline (AalphaC) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), are potent bacterial mutagens and are carcinogenic in rodents. PhIP is mutagenic in the small intestine, but its mutagenicity in the colon, where most human intestinal cancers arise, has not been reported, nor has the mutagenicity of AalphaC. In this study, AalphaC (800 p.p.m.) was fed for 30 and 45 days and PhIP (100 and 400 p.p.m.) was fed for 30, 60 and 90 days to groups of F1 (C57BL/6 x SWR) mice hemizygous for multiple tandem copies of a lacI transgene (the Big Blue mouse) and heterozygous at the endogenous Dlb-1 locus. The mutant frequencies were assayed at Dlb-1 and at lacI in the small intestine and at lacI in the colon. PhIP induced mutations at both loci in the small intestine and induced slightly fewer mutations in the colon. The accumulation of mutations at both loci appears to be linear with both PhIP concentration and duration of exposure and, thus, with dose (concentration x duration). The linear increase with time is in agreement with predictions about the effectiveness of chronic treatment protocols for tests of in vivo mutagenicity. Unlike PhIP, AalphaC induced mutations specifically in the colon and not in the small intestine, thereby showing a dramatic tissue specificity. The rate (mutations/p.p.m. day) was similar to PhIP.

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

CYTOTOXICITY

Baierl T, Drosselmeyer E, Seidel A, Hippeli S. **Comparison of Immunologica effects of fullerene C-60 and raw soot from fullerene production on alveolar macrophages and macrophage like cells in vitro**. Exp Toxicol Pathol 1996;48(6):508-11.
BIOSIS COPYRIGHT: BIOL ABS. RRM Research article bovine carbon 60 fullerene fullerene raw soot immunotoxicity alveolar macrophage toxicodynamics chemotaxis toxicology blood and lymphatics.

Bollo E, Ceppa L, Cornaglia E, Nebbia C, Biolatti B, Dacasto M. **Triphenyltin acetate toxicity: a biochemical and ultrastructural study on mouse thymocytes**. Hum Exp Toxicol 1996;5(3):219-25.
1. Triphenyltin acetate (TPTA) has been shown to exert in vivo a selective toxic effect on the immune system. To assess in vitro possible alterations induced by TPTA exposure, primary cultures of mouse thymocytes were incubated up to 24 h with graded amounts (1-12 microM) of the organotin. 2. The cytotoxic activity has been evaluated with the MTT colorimetric assay, the neutral red (NR) assay and the lactic dehydrogenase (LDH) cellular release. Cell pellets were fixed with 2.5% glutaraldehyde, resin-embedded and ultrathin sections were observed through transmission electron microscopy. 3. After 2 h of incubation, dose-dependent increases of cytotoxicity were observed in thymocytes submitted to MTT and NR tests (up to 41.43% and 18.9%, respectively), while 22 h later this overt effect on cell viability was noticed merely in cells exposed to 12 microM TPTA. Dose-dependent increases of LDH leakage in the culture medium were observed all throughout the study. 4. Morphological investigations revealed features (chromatin condensation, cell membranes fragmentation and formation of membrane bound apoptotic bodies) suggestive of apoptosis. 5. This study indicates that TPTA is cytotoxic to mouse thymocytes: morphologically, the rising of apoptosis is likely to be recognized, as previously reported in different in vitro studies with other immunosuppressive agents as dioxin and corticosteroids.

Dombrink-Kurtzman MA, Bennett GA, Richard JL. **An optimized MTT bioassay for determination of cytotoxicity of fumonisins in turkey lymphocytes**. J AOAC Int 1994;77(2):512-6.
BIOSIS COPYRIGHT: BIOL ABS. In vitro cytotoxicity assays have been performed for detection and quantitation of fumonisins, as possible alternatives for whole animal testing. This study was undertaken to establish optimal in vitro conditions using turkey lymphocytes. Turkey lymphocytes were isolated from peripheral blood by Percoll gradient centrifugation. Cytotoxicity of fumonisin B1 (FB1) and B2 (FB2) was determined by exposing lymphocytes to FB1 or FB2 at concentrations of 0.01-25 mug/mL for 24, 48, or 72 h at 39~C. The MTT bioassay was used to measure cell viability and proliferation. In metabolically active cells, the tetrazolium salt, MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide), was reduced to MTT formazan. Turkey lymphocytes that had been exposed in vitro to FB1 and FB2 for 48 and 72 h showed inhibition of cell proliferation that was dose-dependent. The 50% inhibitory dose for FB1 and FB2 was 0.4-5 mug/mL. Cells exposed to FB1 or FB2 exhibited high levels of cytoplasmic vacuolization and were unable to proliferate, whereas proliferation of control lymphocytes was observed at 48 and 72 h. FB2 was 3- to 4-fold more cytotoxic than FB1.

Giavaresi G, Torricelli P, Fini M, Giardino R. **Pericellular pO2 as an alternative method to test cytotoxicity**. Artif Cells Blood Substit Immobil Biotechnol 1996;24(6):579-86.
BIOSIS COPYRIGHT: BIOL ABS. Pericellular pO2 (PpcO2) was compared with the release of cytoplasmatic enzyme lactate.

Hilger I, Aufderheide M, Knebel JW, Fuchs S, Emura M. **Sensitivity of a hamster lung cell line to**

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

Little MC, Gawkrodger DJ, Macneil S. **Chromiumand nickel-induced cytotoxicity in normal and transformed human keratinocytes: an investigation of pharmacological approaches to the prevention of Cr(VI)-induced cytotoxicity**. Br J Dermatol 1996;134(2):199-207.
The cytotoxicity of chromium (7440473) and nickel (7440020) salts was studied in normal human keratinocytes and the HaCaT keratinocyte cell line. Trivalent and hexavalent chromium salts and nickel-chloride (7718549) were used and cell viability was measured with three different cytotoxicity assays. The three methods used to measure cell viability in this study differed in their ability to detect the cytotoxic effect of hexavalent chromium salts. Even so, all three showed this cytotoxic effect at similar concentrations. The neutral-red dye uptake assay was the most sensitive method used. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide eluted stain assay (MTT-ESTA) was the second most sensitive. Lactate-dehydrogenase (LDH) release into the media was the least sensitive method used. Hexavalent chromium salts were more cytotoxic than trivalent chromium salts in HaCaT cells and in normal human keratinocytes. Salts of a common valency generally showed little difference in cytotoxicity, with only one exception. The authors note that the HaCaT cell line is a useful model for investigating cytotoxicity as it responds to the nickel and chromium compounds in approximately the same manner as normal human keratinocytes and gives relatively consistent responses. The authors conclude that the assessment of keratinocyte metal induced cytotoxicity is a useful in-vitro approach for predicting the effectiveness of preventative agents for chromium and possibly for a wider range of allergens or irritants in contact dermatitis.

Manger RL, Leja LS, Lee SY, Hungerford JM, Hokama Y, Dickey RW, Granade HR, Lewis R, Yasumoto T, Wekell MM. **Detection of sodium channel toxins: directed cytotoxicity assays of purified ciguatoxins, brevetoxins, saxitoxins, and seafood extracts**. J AOAC Int 1995;78(2):521-7.
BIOSIS COPYRIGHT: BIOL ABS. Neuroblastoma cells in culture were used to detect sodium channel-

specific marine toxins based on an end-point determination of mitochondrial dehydrogenase activity. The assay responds in a dose-dependent manner to ciguatoxins, brevetoxins, and saxitoxins, and delineates the toxic activity as either sodium channel enhancing or sodium channel blocking. The assay responds rapidly to sodium channel activating toxins, allowing dose dependent detection in 4 to 6 h. Brevetoxins can be detected at 250 pg, and purified ciguatoxins are detected in the low picogram and subpicogram levels. The results obtained from cell bioassay of ciguatoxic finfish extracts correlates with those obtained from mouse bioassays. Sodium channel blocking toxins can also be detected with an approximate sensitivity of 20 pg in 24 to 48 h. This cell-based technique is simple, sensitive, demonstrates potential as an alternative to animal testing for sodium channel activating and blocking toxins, and can be automated.

Oulmi Y, Braunbeck T. **Toxicity of 4-chloroaniline in early life-stages of zebrafish (Brachydanio rerio): I. cytopathology of liver and kidney after microinjection**. Arch Environ Contam Toxicol 1996;30(3):390-402.
In addition to survival and hatching parameters, cytological alterations in liver and kidney of 4- and 6-d old zebrafish larvae (Brachydanio rerio) following single microinjection of fertilized eggs at the germ-ring stage with 5, 12.5, and 25 ng 4-chloroaniline/egg were investigated by means of electron microscopy. Whereas survival remained unaffected, microinjection with 4-chloroaniline disturbed hatching of larvae. Hatching was delayed by microinjection of 12.5 ng 4-chloroaniline/egg and above when compared to controls. Cytological investigations revealed ultrastructural changes in both liver and kidney in a dose- and time-dependent fashion. In the liver, major cytopathological changes included fenestration, fragmentation, and vesiculation of the rough endoplasmic reticulum, proliferation of atypical mitochondria, and atypical lysosomes. Furthermore, myelin whorls, lipid inclusions, and cholesterol crystals were increased, whereas glycogen stores were reduced. Renal tubular cells displayed altered brush borders, proliferation of nucleoli, atypical mitochondria, fenestrated, fragmented, and vesiculated RER cisternae, as well as giant lysosomes. Most of these effects indicate cellular dysfunction (e.g., disturbance of lipid metabolism in the liver), whereas others illustrate general cellular stress-responses to chemical aggression. Comparisons of results with those of previous studies based on conventional fish exposure prove the suitability and sensitivity of microinjection bioassays with zebrafish eggs as an alternative to conventional early life-stage tests.

Schweiki H, Schmalz G. **Toxicity parameters for cytotoxicity testing of dental materials in two different mammalian cell lines**. Eur J Oral Sci 1996;104(3):292-9.
The present study compares three specific toxicity parameters for cytotoxicity testing of chemically different dental materials. Two glass ionomer cements, a zinc phosphate cement, and a composite material were used to evaluate the sensitivity of three assays: two viability assays, the MTT assay and the quantifiable neutral red assay, and a proliferation assay based on the determination of the total protein content of a cell culture. The colorimetric assays were carried out using transformed mouse fibroblasts (L-929 cells) and fibroblasts derived from biopsies of normal human gingiva. In most cases, all colorimetric assays detected much weaker cytotoxic responses, if any, in gingival fibroblasts than in L-929 cells. The viability assays indicated cytotoxicity of the extracts to two glass ionomer cements in L-929 cells when the materials were set at 0% relatively humidity for 24 h. The severe cytotoxicity of the zinc-phosphate cement in both viability assays was less influenced by the setting conditions. The

cytotoxicity of the composite material was most pronounced in the neutral red assay. In general, both the MTT assay and the neutral red assay were more sensitive than the colorimetriC Proliferation assay. These assays can be performed very effectively; only few cells are needed for rapid, reliable and inexpensive screening purposes of a large number of samples in a short time. Automated processing with a microplate reader after non-radioactive labeling of the cells and subsequent automated analyses of original data, with no need for sophisticated and expensive equipment, are additional advantages of the systems.

Slamenova D, Ruppova K, Gabelova A, Wsolova L. **Evaluation of mutagenic and cytotoxic effects of sodium fluoride on mammalian cells influenced by an acid environment**. Cell Biol Toxicol 1996;12 (1):11-7.
The mutagenic activity of sodium fluoride at reduced pH was studied in the V79/HGPRT system. Statistical analysis of the results of mutagenicity testing suggests that, despite its high toxicity, sodium fluoride has no mutagenic effects at reduced pH on hamster V79 cells. Short-term treatment of cells with sodium fluoride at reduced pH inhibits growth activity of cells as well as synthesis of pulse-labeled nascent DNA and cumulative RNA synthesis and proteosynthesis. From the results of this study we suggest that an acid environment which supports formation of hydrogen fluoride increases toxic but not mutagenic potencies of sodium fluoride.

Tillner J, Winckler T, Dingermann T. **Developmentally regulated promoters from Dictyostelium discoideum as molecular markers for testing potential teratogens**. Pharmazie 1996;51(11):902-6.
BIOSIS COPYRIGHT: BIOL ABS. Already very early in the course of the development of new pharmaceutically relevant drugs toxicological tests are most important. In addition to acute and chronic toxicity the estimation of the teratogenic potential is rather crucial. We have recently shown that the eukaryotic microorganism Dictyostellium discoideum is a useful organism to test the cytotoxicity of chemical compounds. Since D. discoideum is competent of undergoing both vegetative growth and development, further investigations were aimed to establish a D. discoideum-based test system which could predict possible interference of drugs with developmental programs. We developed a method which allows to detect and to quantify effects of possible teratogens on D. discoideum development. This method is based on different transgenic D. discoideum strains, each carrying a bacterial lacZ gene under the control of a distinct developmentally regulated D. discoideum promoter. Here we describe the effects of the known teratogenic compound valproic acid (VPA) on this system.

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

okadaic acid content of 16 naturally contaminated samples measured by the MTT cytotoxicity assay and by an ELISA.

DERMAL TOXICITY

Ahmed S, Imai T, Otagiri M. **Evaluation of stereoselective transdermal transport and concurrent cutaneous hydrolysis of several ester prodrugs of propranolol: mechanism of stereoselective permeation**. Pharm Res 1996;13(10):1524-9.
CBAC COPYRIGHT: CHEM ABS The purpose of this study was to evaluate the stereoselective permeation and concurrent cutaneous hydrolysis of a series of ester prodrugs of propranolol (PL). In vitro studies were performed across full-thickness, stripped and diisopropylfluorophosphate (DFP) treated skins of hairless mouse with flow-through diffusion cells at 37.degree.. The permeability coeffs. (Kp), which were dependent on partition coeffs. (PC), of all the prodrugs were markedly increased compared to the parent drug. In full-thickness skin, the (R) caproyl-PL (CR-PL) showed the highest Kp, which was about 52-fold greater than that of PL. Most of the more lipophilic prodrugs showed stereoselectivity in Kp (R > S). All the prodrugs underwent stereoselective hydrolysis (R > S) during penetration. The prodrugs which showed stereoselectivity in permeation were comparatively lipophilic and showed great difference in hydrolysis percentages between the enantiomers. Permeation studies with stripped skin revealed that prodrugs were more permeable across stratum corneum compared to PL, whereas reverse was happened across viable skin. Although CR-PL showed high stereoselectivity in permeation across full-thickness skin and underwent higher percent of concurrent stereoselective cutaneous hydrolysis, the prodrug showed no stereoselectivity in permeation across DFP, an esterase inhibitor, treated skin and the concurrent cutaneous hydrolysis was also stopped. Lipophilic prodrugs may readily pass the stratum corneum but may not be able to penetrate so easily through the deeper tissues. Unlike the (S) isomers, the (R) isomers of lipophilic prodrugs almost completely converted to propranolol in epidermis and can easily pass through the dermis layer, resulting in stereoselective penetration.

Baynes RE, Brownie C, Freeman H, Riviere JE. **In vitro percutaneous absorption of benzidine in complex mechanistically defined chemical mixtures**. Toxicol Appl Pharmacol 1996;141(2):497-506.
CBAC COPYRIGHT: CHEM ABS Since humans are more likely to be exposed to chem. mixts. than to a single chem., a program was developed in these labs. to examine the cumulative effect of complex mixts. on percutaneous absorption of important toxicants such as benzidine. In this investigation, a mixt. is defined as a phys. combination consisting of a marker chem. and several other chems., each of which can have independent and/or synergistic effects on dermal penetration and absorption of the marker chem. Ten mixts., consisting of a marker chem. (benzidine, B), a solvent (acetone, A or DMSO, D), a surfactant (0 or 10% sodium lauryl sulfate, SL), a vasodilator (0 or 180 mug Me nicotinate, M), and a reducing agent (0 or 2% SnCl2) were employed in this study. Isolated perfused porcine skin flaps (IPPSFs), which have proven to be a suitable in vitro model for assessing dermal absorption and toxicity, and flow-through diffusion cell systems were utilized. The extent of benzidine absorption in skin sections dosed with either B + A (0.94% dose) or B + D (1.01% dose) was similar to that when

IPPSFs were dosed with either B + A (0.54% dose) or B + D (1.31% dose). However, flux vs. time profiles were different when the two in vitro methods were compared. For mixts. contg. (1) DMSO only or acetone only or (2) solvents contg. SL + M, benzidine absorption was enhanced when compared with other mixts. Compared to acetone, DMSO appears to enhance dermal penetration of benzidine in most of the mixts. Compared to other mixts. evaluated, SnCl2 inhibited benzidine absorption irresp. of solvent present. SnCl2 also appears to inhibit benzidine penetration in DMSO mixts. contg. SL, only, but not in acetone mixts. It is proposed that chem.-chem. interactions between benzidine and SnCl2 may be inhibiting benzidine absorption and chem.-biol. interactions between M + SL and skin may be enhancing benzidine absorption. Across all mixts., max. obsd. benzidine absorption was almost 3% of the topical dose over 8 h, but max. penetration was 22% over the same time period which would suggest a potential for greater systemic exposure over longer time frames. This work underscores the need to study potentially toxic chems. in mixt. exposure scenarios since the interactions obsd. would confound risk assessment based on single chem. data.

Bosman IJ, Lawant AL, Avegaart SR, Ensing K, De Zeeuw RA. **Novel diffusion cell for in vitro transdermal permeation, compatible with automated dynamic sampling**. J Pharm Biomed Anal 1996;14(8-10):1015-23.
The development of a new diffusion cell for in vitro transdermal permeation is described. The so-called Kelder cells were used in combination with the ASPEC system (Automatic Sample Preparation with Extraction Columns), which is designed for the automation of solid-extractions (SPE). Instead of SPE columns, 20 Kelder cells were placed in the racks. This allowed automatic sampling of up to 20 cells for 24 h in a dynamic mode. The cells consist of an inlet compartment, a donor compartment and a receptor compartment. The size and the depth of the inlet compartment were important to avoid entrapment of air bubbles in the receptor compartment. The Kelder cells mimic blood flow beneath the skin by replacement of the permeating drug every 2 min. Hence sink condition are more easily maintained than with the static Franz diffusion cell. The performance of the cells was tested with permeation experiments using atropine as a model drug permeating through an artificial membrane (Silastic). The use of this skin model minimized the variability in permeation of atropine as compared with human skin.

Bronaugh RL. **In vitro viable skin model**. Pharm Biotechnol 1996;8:375-86.
CBAC COPYRIGHT: CHEM ABS A review with 13 refs. Absorption and metab. of topically applied drugs by skin can be readily measured by using in vitro techniques. A thin prepn. of skin simulating the barrier layer is prepd. and placed in a flow-through diffusion cell. A physiol. buffer maintains the viability of skin for at least 24 h. The investigator can obtain information related to the absorption of the parent compd., as well as information on its biotransformation during the absorption process.

Effendy I, Weltfriend S, Kwangsukstith C, Singh P, Maibach HI. **Effects of all-trans retinoic acid and sodium lauryl sulphate on the permeability of human skin in vitro**. Br J Dermatol 1996;135(3):428-32.
Recent in vivo investigations have shown that pretreatment with topical all-trans retinoic acid (RA) may diminish the skin response to sodium lauryl sulphate (SLS). This study evaluated the permeation of SLS through human skin after pretreatment with RA, and vice versa, by in vitro methods. The permeability coefficient of SLS (3.24 +/- 0.21 x 10(3) cm/h) and the 24-h cumulative amount of SLS (3.41 +/- 0.6% of dose applied) permeating RA-pretreated skin did not differ significantly from those across untreated

skin (control) (P > 0.05). In contrast, the permeability coefficient of RA (0.23 +/- 0.05 x 10(3) cm/h) and its 24-h cumulative amount (0.37 +/- 0.05% of dose applied) penetrating SLS-pretreated skin were significantly greater than those permeating untreated skin (P < 0.05). Thus, an increase in RA penetration was induced by SLS pretreatment; however, pretreating the skin with RA did not inhibit the percutaneous permeation of SLS. Based on previous in vivo findings where RA reduced skin reactions to SLS, one would speculate that RA pretreatment may decrease SLS penetration. However, these penetration data do not necessarily uphold this presumption. Perhaps, other interactions between the substances and the skin, e.g. at cellular levels, may be responsible for the differing skin responses.

Fang J, Huang Y, Wu P, Tsai Y. **Transdermal iontophoresis of sodium nonivamide acetate I. Consideration of electrical and chemical factors**. Int J Pharm 1996;143(1):47-58.
CBAC COPYRIGHT: CHEM ABS Transdermal iontophoresis is a process which enhances skin permeation of ionized species by an elec. field as driving force. The aim of this present study was to investigate the transdermal iontophoresis of a newly designed capsaicin deriv., sodium nonivamide acetate (SNA). Studies of elec. and physicochem. factors acting on the kinetics of in vitro iontophoresis were performed. Iontophoresis increased the transdermal penetration flux of SNA as compared to the passive diffusion in this study. Several application modes which possessed the same elec. energy had been researched. The iontophoretic flux of SNA increased following the decrease of donor buffer pH values. This trend could be due to the physiol. property of skin and electro-osmotic flow presented. Comparing the various application modes, the discontinuous on/off cyclic current mode showed higher penetration capacity than did continuous mode which was due to the intensity of effective current which would not decay for on/off cyclic application of iontophoresis. The result of the present study is particularly helpful in the development of a SNA transdermal iontophoretic delivery system.

Fares HM, Chatterjee S, Hayward M. **In vitro permeation and irritation of benzoyl peroxide-containing products**. Int J Pharm 1996 May 14;133:215-22.
IPA COPYRIGHT: ASHP Two fast and reproducible in vitro methods that measure permeation of benzoyl peroxide from formulations and use Franz-type diffusion cells with silicone sheeting in 1 model and cultured human epidermis in the other as barriers and another model that uses cell viability to measure irritation of benzoyl peroxide formulations are described and used to evaluate 2.5-10% vanishing and tinted benzoyl peroxide gels. The release profiles, rates of permeation, and diffusion coefficients from both models were similar. Cell viability was linearly related to the release rate of benzoyl peroxide.

Goffin V, Pierard GE. **Corneosurfametry and the compromised atopic stratum corneum**. Arch Dermatol Res 1996;288(8):489-91.

Grandolfo M, Pipoli M, Foti C, Bonamonte D, Rigano L, Vena GA, Angelini G. **Influence of vehicle on patch test response to nickel sulfate**. Contact Dermatitis 1996;35(3):173-4.
BIOSIS COPYRIGHT: BIOL ABS. RRM Note research article human allergy dermatology contact dermatitis patch test nickel sulfate patch test response to nickel sulfate percutaneous penetration enhancers contact allergy cosmetics integumentary system disease diagnostic method influence of vehicle.

Harrison JE, Groundwater PW, Brain KR, Hadgraft J. **Azone induced fluidity in human stratum corneum: Fourier transform infrared spectroscopy investigation using the perdeuterated analog**. J Control Release 1996 Sep; 41:283-90.
IPA COPYRIGHT: ASHP The effect of 2 levels of laurocapram (Azone) treatment on human stratum corneum (SC) lipid fluidity was investigated by the use of Fourier transform-IR spectroscopy and its perdeuterated analog; SC lipid fluidity was monitored in terms of the degree of organization of the lipid acyl chains and in terms of the rate of motion of the lipid chains within the ordered environment. At the lower level, results indicated that D-laurocaprone did induce disordering of the lipid acyl chains by the introduction of gauche conformers above the SC lipid gel to liquid crystalline phase transition midpoint (Tm). The rate of motion of the chains was increased at lower temperatures indicating the D-laurocapram did affect 1 aspect of SC lipid fluidity below Tm. At the higher level of D-laurocapram treatment the SC lipid fluidity was increased with respect to both aspects of fluidity examined, both above and below the SC lipid phase transition. It was concluded that this action may suggest how the penetration of molecules through the skin is enhanced by laurocapram.

Harrison JE, Watkinson AC, Green DM, Hadgraft J, Brain K. **Relative effect of Azone and Transcutol on permeant diffusivity and solubility in human stratum corneum**. Pharm Res 1996 Apr;13:542-6.
IPA COPYRIGHT: ASHP To analyze the mechanism of the enhancement of percutaneous penetration demonstrated by laurocapram (Azone) and ethoxydiglycol (Transcutol), enhancer induced changes in the diffusivity and solubility of 4-cyanophenol in human stratum corneum were monitored using Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy and compared to the gross effects of the enhancers on flux as measured using simple Franz-type diffusion cells. Both enhancers increased the flux of 4-cyanophenol across human skin in vitro by a factor of approximately 2. It was also demonstrated by ATR-FTIR that these enhancers were likely to exert their effects by different mechanisms. It is probable that laurocapram reduces the diffusional resistance of the stratum corneum and that ethoxydiglycol increases the solubility of the penetrant in this barrier. It was concluded that the application of ATR-FTIR spectroscopy to these enhancing systems will allow the mechanisms of the observed enhancements to be probed in greater depth.

Holliday MR, Dearman RJ, Corsini E, Basketter DA, Kimber I. **Selective stimulation of cutaneous interleukin 6 expression by skin allergens**. J Appl Toxicol 1996;16(1):65-70.
Epidermal cells, both keratinocytes and Langerhans cells, are able to synthesize and secrete a variety of cytokines, many of which influence or are essential for the induction of skin sensitization and the elicitation of local inflammatory reactions. It has been proposed that it may prove possible to distinguish between contact allergens and skin irritants as a function of differential induction or upregulation of epidermal cytokine expression. In the present study we have addressed this by examination of the local cutaneous production of interleukin 6 (IL-6) following topical exposure of mice to oxazolone, a potent contact allergen, or to benzalkonium chloride (BZC), a skin irritant that is considered not to have a significant potential to cause skin sensitization. Both oxazolone and BZC could induce the production of IL-6 as measured by enzyme-linked immunosorbent assay in homogenates prepare from treated skin. However, when these chemicals were applied at concentrations that resulted in equivalent cutaneous inflammatory responses, based on induced oedema, only oxazolone provoked the production in skin of IL-6. Moreover, under these conditions exposure only to oxazolone resulted in the secretion by draining

lymph node cells of measurable concentrations of this cytokine. These data suggest that the ability of oxazolone to stimulate local IL-6 production is not secondary simply to the induction of local inflammatory responses. As such, the results support the possibility that skin allergens and skin irritants may stimulate variable patterns of epidermal cytokine production.

Hostynek JJ, Magee PS, Maibach HI. **QSAR predictive of contact allergy: scope and limitations**. Curr Probl Dermatol 1996;25:18-27.

Jain GK, Sharma AK, Agrawal SS. **Transdermal controlled administration of verapamil enhancement of skin permeability**. Int J Pharm 1996 Mar 22;130:169-77.
IPA COPYRIGHT: ASHP A matrix transdermal system containing verapamil hydrochloride was prepared using polyvinyl alcohol and povidone (PVP; polyvinylpyrrolidone), and the effect of a penetration enhancer, (+)-limonene (d-limonene), on the rate and amount of drug permeated across skin was studied in vitro. Drug permeation was enhanced by (+)-limonene. The permeation rate followed approximately zero order kinetics. At concentrations of 2 and 5%, (+)-limonene did not affect permeation significantly, but at 20%, the effect was significant. Formulations containing the enhancer had lower lag times. This effect was more pronounced in those containing higher concentrations of enhancer. Permeation increased extensively with the use of both polyvinyl alcohol and povidone instead of either polymer alone.

Ji XF, Peng QN, Liu GJ, Zhou Y. **[Effect of enhancers on the timolol transdermal therapeutic system]**. Zhongguo Yaoke Daxue Xuebao 1996;27(1):6-9. (Chi)
IPA COPYRIGHT: ASHP A reliable UV method for the determination of timolol permeation through rat skin in vitro was developed and used to evaluate the effect of laurocapram (azone), oleic acid, propylene glycol (1,2-propylene glycol), and 2-pyrrolidone (alpha-pyrolidone) on timolol permeation. Laurocapram was superior to oleic acid that was superior to propylene glycol that was superior to 2-pyrrolidone. Mixed enhancers accelerated penetration of timolol.

Kashima R, Oyake Y, Okada J, Ikeda Y. **Improved ex vivo/in vitro lymph node cell proliferation assay in guinea pigs for a screening test of contact hypersensitivity of chemical compounds**. Toxicology 1996;114(1):47-55.
Simple and efficient ex vivo/in vitro screening systems for contact allergens are developed for alternative to conventional animal tests. We have previously proposed an ex vivo/in vitro proliferation assay as a first stage screening method with advantages over existing alternatives, using lymph node cells (LNC) from sensitized guinea pigs of the Hartley strain. In this study, we have first confirmed, by histochemical analysis using in vivo bromodeoxyuridine (BrdU) and pyronin staining, that the ex vivo/in vitro LNC proliferation reflects in vivo response of lymph nodes to contact allergens. Furthermore, to improve the LNC assay, we then have investigated several experimental conditions for their influences on the LNC assay, demonstrating that, (1) the subscapular and the cervical LNC responded highly to contact allergens, (2) among three cervical lymph nodes the superficial dorsal cervical lymph nodes were the most reactive, (3) several vehicles alone used for animal sensitization exhibited little influence on the LNC proliferation, (4) employment of stimulation index offset the inter-batch fluctuation of the LNC proliferation in the control animals as baseline proliferation. Under optimized experimental

conditions as above, experimentally determined stimulation indexes of several contact allergens correlated well with their sensitizing potential estimated by conventional animal tests. Therefore, the ex vivo/in vitro LNC proliferation assay should be a simple and efficient alternative to conventional guinea pig testings including the guinea pig maximization test (GPMT).

Kirjavainen M, Urtti A, Jaeaeskelaeinen I, Suhonen TM, Paronen P, Valjakka-Koskela R, Moenkkoenen J. **Interaction of liposomes with human skin in vitro - the influence of lipid composition and structure**. Biochim Biophys Acta 1996;1304(3):179-89.
CBAC COPYRIGHT: CHEM ABS Liposomes have been suggested as a vehicle for dermal and transdermal drug delivery, but the knowledge about the interaction between lipid vesicles and human skin is poor. Therefore, we visualized liposome penetration into the human skin by confocal laser scanning microscopy (CLSM) in vitro. Liposomes were prepd. from phospholipids in different compns. and labeled with a fluorescent lipid bilayer marker, N-Rh-PE (L-alpha-phosphatidylethanolamine-N-lissamine rhodamine B sulfonyl). Fluorescently labeled liposomes were not able to penetrate into the granular layers of epidermis. However, the fluorescence from liposome compns. contg. DOPE (dioleylphosphatidyl ethanolamine) was able to penetrate deeper into the stratum corneum than that from liposomes without DOPE. Pretreatment of skin with unlabeled liposomes contg. DOPE or lyso-phosphatidyl choline (lyso-PC) enhanced the subsequent possible enhancer activity, while most liposomes did not show such enhancement. Resonance energy transfer (RET) and calcein release assay between stratum corneum lipid liposomes (SCLLs) and the phospholipid vesicles suggested that the liposomes contg. DOPE may fuse or mix with skin lipids in vitro and loosen the SCLL bilayers, resp. Among the factors not affecting stratum corneum penetration were: neg. charge, cholesterol inclusion and acyl chain length of the phospholipids. In conclusion, fusogenicity of the liposome compn. appears to be a prerequisite for the skin penetration.

Kontturi K, Murtomaki L. **Mechanistic model for transdermal transport including iontophoresis**. J Control Release 1996 Sept;41:177-85.
IPA COPYRIGHT: ASHP A mechanistic model to describe the transdermal transfer of drugs, including iontophoresis, is presented; in the model, flux is divided into the contributions through the lipid matrix and through aqueous pores. During iontophoresis, only flux through aqueous pores is enhanced because the relative permettivity of the lipid matrix is too low to allow the existence of free ions that could work as current carriers. The transfer through the lipid matrix is assumed to take place in 3 steps: 1) partition at the skin/reservoir interace; 2) diffusion through the lipid matrix; and 3) desorption at the stratum cornem/epidermis interface. Simulations to the present model agreed with experimental results in the literature.

Krasteva M, Pegeut-Navarro J, Moulon C, Courtellemont P, Redziniak G, Schmitt D. **In vitro primary sensitization of hapten-specific T cells by cultured human epidermal Langerhans cells: a screening predictive assay for contact sensitizers**. Clin Exper Allergy 1996;26(5):563-70.
As part of an investigation into possible methods for the in-vitro identification of skin sensitizing chemicals in humans, the ability of several contact sensitizers to cause in-vitro primary sensitization of autologous T-cells with cultured human Langerhans cells as antigen presenting cell (APC) was tested. Tritiated thymidine incorporation analysis was performed to determine T-cell proliferation induced by haptens in cultured human Langerhans cells. Allergens tested included the strong contact allergens 2,4,6-

trinitrobenzenesulfonic-acid (2508192) (TNBS), p-phenylenediamine (106503) (PPDA), and fluorescein-isothiocyanate (3326327) (FITC); the low contact sensitizing agents citronellal (106230), hydroxycitronellal (107755), and coumarin (91645); and the irritant sodium-dodecyl-sulfate (151213) (SDS). T-cell responses were were produced by the strong allergens in most trials, while SDS did not produce T-cell proliferation in any trial. Weak sensitizers produced lymphoproliferative responses in 1/12 (coumarin), 0/10 (citronellal), and 2/8 (hydroxycitronellal) trials. The authors conclude that the in-vitro human T-cell sensitization model proved effective in discriminating between strong contact sensitizers and weak contact sensitizers and irritants, and could be used as an assay to screen out strong contact allergens.

Krishna R, Pandit JK. **Carboxymethylcellulose-sodium based transdermal drug delivery system for propranolol**. J Pharm Pharmacol 1996;48(4):367-70.
Propranolol, a beta-adrenoceptor blocker, suffers from a high degree of first-pass metabolism resulting in very low bioavailability (< 10%) following administration with conventional oral formulations. To circumvent this significant therapeutic hurdle, we formulated a carboxymethylcellulose-sodium (CMC-Na) based transdermal system for propranolol and evaluated the patch for its in-vitro and in-vivo performance. In-vitro permeation studies using the excised hair-free rat skin model resulted in 66.54% permeation at the end of 24 h in a modified Franz diffusion cell. This zero-order permeation profile was characterized by a drug permeation rate of 52.87 +/- 11.63 micrograms cm-2 h-1. Skin irritation studies in rats (n = 5), evaluated for flare-and-wheal with respect to a formalin control, indicated that the drug-containing patch evoked only a mild response over a 7-day period. Preliminary in-vivo studies in male albino rabbits (n = 3), indicated that plasma drug levels averaged 11.75 +/- 3.40 ng mL-1 in a 24-h study period before patch removal.

Macpherson SE, Barton CN, Bronaugh RL. **Use of in vitro skin penetration data and a physiologically based model to predict in vivo blood levels of benzoic acid**. Toxicol Appl Pharmacol 1996; 140(2): 436-43.
A physiologically based pharmacokinetic (PB PK) model was developed to predict plasma levels of benzoic acid (BA) in the hairless guinea pig after topical exposure at three finite dose levels. The PB PK model consisted of four compartments: (1) rapidly perfused tissues; (2) slowly perfused tissues; (3) liver, representing the route of elimination of BA from the plasma after biotransformation to hippuric acid; and (4) plasma. The predictive capacity of the PB PK model was assessed by comparing plasma BA levels measured experimentally with those predicted by the model. The percutaneous absorption of finite doses of BA in the model was described by a transdermal input function, which was derived from in vitro percutaneous absorption studies in which viable hairless guinea pig skin in flow-through diffusion cells was exposed to BA. Physiological parameters used in the model were calculated from previously published values. Biochemical parameters, including partition coefficients and metabolic constants, were measured experimentally in vitro. The PB PK model predictions were generally in good agreement with measured plasma levels for each of the dose levels studied. The predicted plasma BA levels and the measured values were closer for the highest dose (120 microg/cm2) than for either of the other two doses used (12 and 40 microg/cm2). The effects of optimizing the metabolic constants and the transdermal input function parameters on the predicted curve shape and fit to that of the measured plasma BA levels were assessed. Varying the transdermal input parameters produced closer agreement

between predicted and measured values.

Matschiner S, Neubert R, Wohlrab W, Matschiner F. **Influence of ion pairing on ex vivo penetration of erythromycin into sebaceous follicles**. Skin Pharmacol 1996;9(4):270-3.
CBAC COPYRIGHT: CHEM ABS The results of the penetration of an ion pair composed of erythromycin (ERY) and octadecansulfonate (OS) obtained in a multilayer membrane model were evaluated with excised human skin. The amt. of ERY penetrating into sebaceous follicles of freshly excised human skin was measured using [N-methyl-14C]erythromycin. The ex vivo penetration of the ion pair ERY/OS into the sebaceous follicles was obsd. to be doubly enhanced compared with the penetration of the ERY base. The model was shown to be suitable for predicting in vivo penetration of anti-acne formulations into sebaceous glands.

Menczel E. **Assessment of delipidization as an enhancing factor in percutaneous penetration**. Curr Probl Dermatol 1996;22:189-94.
CBAC COPYRIGHT: CHEM ABS Effects of delipidization of skin on percutaneous penetration of drugs are discussed. Examples used are percutaneous penetration of estrogens, and lidocaine after delipidization of skin with solvents such as ethanol, benzene, or trichloroethane.

Mikulowska A, Andersson A. **Sodium lauryl sulfate effect on the density of epidermal Langerhans cells. Evaluation of different test models**. Contact Dermatitis 1996;34(6):397-401.
The effect of different test models for sodium lauryl sulfate (SLS)-induced irritant contact dermatitis on epidermal Langerhans cells (LC) numbers was examined. Finn Chambers, 8 and 12 mm, containing 15 and 34 or 50 microliters, respectively, of 1% aq. solution of SLS were applied to human forearm skin for 48 h as single or repeated application. The results showed a clear difference between the effects with the 2 chamber sizes. The effect of the 8-mm chambers could result in increased, unchanged or decreased LC numbers, while 12-mm chambers always produced a decrease. These results seem to explain, at least partly, the discrepant results reported from various laboratories.

Neubert R, Schmalfuss U, Wohlrab W, Huschka C. **[Active substance penetration in the skin and its modulation]**. Pharm Ztg 1996 Apr 25;141:11-16, 18, 21-3. (Ger)
IPA COPYRIGHT: ASHP The mechanisms of active substance penetration of the skin and the uses of modulators, including enhancers, pro drugs, ion pair formation, and phono- and iontophoresis to optimize skin permeability, along with reducers for drug localization to minimize side effects, are presented.

Niazy EM. **Differences in penetration enhancing effect of Azone through excised rabbit, rat, hairless mouse, guinea pig and human skins**. Int J Pharm 1996 Mar 22;130:225-30.

Noz KC, Bauwens M, Van Buul PP, Vrolijk H, Schothorst AA, Pavel S, Tanke HJ, Vermeer BJ. **Comet assay demonstrates a higher ultraviolet B sensitivity to DNA damage in dysplastic nevus cells than in common melanocytic nevus cells and foreskin melanocytes**. J Invest Dermatol 1996;106(6):1198-202.
We used the single cell gel electrophoresis assay (comet assay) to study ultraviolet B (UVB)-induced

DNA damage in pigment cells. This assay detects DNA damage, mainly DNA strand breaks and alkali labile sites in the DNA molecule. We studied the effect of biologically relevant doses (comparable to 2-3 MED (minimal erythemal dose) for in vivo irradiated full-thickness skin) of monochromatic UVB light of 302 nm on cultured melanocytes derived from foreskin, common melanocytic nevi, and dysplastic nevi. We were able to demonstrate a linear dose-response relationship between UV dose and the migration coefficient of the comet tail in all three types of pigment cells. Nevus cells originating from dysplastic nevi showed the highest sensitivity to UVB.

Ohara N, Takayama K, Isowa K, Nagai T. **[Optimal condition of combined use of penetration enhancer and applied heat for the percutaneous absorption of ketoprofen]**. Yakuzaigaku 1996; 56 (1):40-8 . (Jpn)
IPA COPYRIGHT: ASHP To investigate the optimal conditions of penetration enhancer, ethyl alcohol concentration, and applied heat for ketoprofen percutaneous absorption from hydrogels, a computer optimization technique based on response surface methodology using (+)-limonene (d-limonene) or oleic acid as penetration enhancers was employed and pharmacokinetic parameters in rats were evaluated. The penetration rate of ketoprofen under optimal conditions obtained in the system containing (+)-limonene was excellent compared with the system of oleic acid. However, skin damage with limonene was significantly greater than that with oleic acid.

Perkins MA, Osborne R, Johnson GR. **Development of an in vitro method for skin corrosion testing**. Fundam Appl Toxicol 1996;31(1):9-18.
National and international regulations require that chemicals must be properly classified, labeled, packaged, and transported based on their ability to damage or destroy tissue, e.g., skin. Traditionally, skin corrosion assessments were based on tests involving topical application of test substances to the skin of rabbits. In the present work, an in vitro skin corrosion test based on the use of reconstructed human skin cultures was developed as a potential replacement for in vivo rabbit skin tests for corrosion. In the in vitro method, test substances were applied topically to the stratum corneum surface of human skin cultures. Skin culture damage or cytotoxicity was measured as decreased 3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide (MTT) vital dye metabolism. In time-course experiments, the time (in minutes) of test material exposure eliciting a 50% reduction of MTT metabolism (i.e., t50 value) was calculated. Using this method we evaluated 24 chemicals and found that the 9 corrosive chemicals were accurately distinguished from 15 strong.

Pinazo A. **Effect of surfactant structure on diffusion through a collagen membrane**. Colloids Surf B 1996;8(1-2):63-72.
CBAC COPYRIGHT: CHEM ABS The objective of this paper was to study the behavior of a collagen membrane when diffusing surfactants through it. The tests were performed using sodium dodecyl sulfate, alkyldimethylamine oxides and alkyldimethylamine betaines. When using mixts. of these compds., the total amt. of diffused surfactant has a min. which corresponds to the range of compns. with a min. skin irritation potential according to std. irritability values. FT-IR spectroscopy was applied to evaluate the water content of diffusion collagen membranes. Collagen membranes were useful for studying both the diffusion phenomena of surfactants and their mixts., and the variation of the water contents.

Qiao GL, Brooks JD, Baynes RE, Monteiro-Riviere NA, Williams PL, Riviere JE. **The use of mechanistically defined chemical mixtures (MDCM) to assess component effects on the percutaneous absorption and cutaneous disposition of topically exposed chemicals. I. Studies with parathion mixtures in isolated perfused porcine skin**. Toxicol Appl Pharmacol 1996;141(2):473-86.
Recently, attention has been directed to the risk assessment of cutaneous exposure to chemical mixtures rather than to only a single compound since this is the exposure scenario in the environment, residence, and work place. Using acetone or dimethylsulfoxide (DMSO) (80% in water) as a vehicle, percutaneous absorption and cutaneous disposition of parathion (PA) were studied following PA (40 microg/cm2) dosing on isolated perfused porcine skin as mechanistically defined chemical mixtures (MDCM) consisting of the surfactant sodium lauryl sulfate (SLS), the rubefacient methyl nicotinate (MNA), and the reducing agent stannous chloride (SnCl2). A full 2 x 4 factorial design was used to asses treatment effects and potential interactions. More radiolabel was absorbed with DMSO than with acetone albeit an earlier peak flux time but lower peak flux was observed with acetone than with DMSO. The absorption flux rate profiles with DMSO continued increasing but bipeak-featured profiles were observed with acetone. SLS enhanced PA absorption with both DMSO and acetone. The presence of MNA in both vehicles blunted the absorption rate curves without significantly changing total absorption. SnCl2 blocked PA absorption and increased residue level on the skin surface and in the stratum corneum (SC). The venous flux profiles were mixture-dependent and highly reproducible within treatment groups. Higher level interactions were also noted. This study indicated multiple levels of interactive effects on PA absorption which must be incorporated into any effort to identify critical mechanisms which affect risk assessment of topically exposed mixtures. It was suggested that the chemicals selected in a topically applied mixture may have significant effects on the penetration/distribution pattern and percutaneous absorption profile of a toxicant/drug in the mixture. The MDCM approach may be useful in a screening or triage approach to identify mixture components which affect marker chemical absorption as well as identify potential mechanisms which deserve further attention. Risk assessment efforts could then be focused on those mixtures, containing these critical components, which would be expected to have the greatest penetration and absorption.

Rajadhyaksha VJ, Sehgal S. **Compositions and methods for inducing dermal analgesia**. PCT Int Appl PATENT NO 96 33706 10/31/96 (Pharmetrix, Inc).
CBAC COPYRIGHT: CHEM ABS Compns. for the topical delivery of anesthetic agents, are emulsified creams which contain at least one, and preferably two or more anesthetic agents, a cell envelope disordering compd., a lipophilic permeation enhancer, a polyoxyethylene ether, a solubilizing agent, a self-emulsifying agent, and water. These multi-component systems effectively allow the penetration of the active agents to produce both a rapid onset and longer duration of anesthesia for the tissue to which the compn. is applied, preferably under occlusion. Propylene glycol (2.5 %) was added to water (65 %) with stirring at room temp. A self-emulsifying agent, Tefose-63 (20 %) was melted in triethylene glycol monomethyl ether (2.5 %) and 2-octyldodecanol (2.5 %) by gentle warming to 55.degree.. To the melt was added 4-decyloxazolidinone (2.5 %), followed by prilocaine base (2.5 %) and lidocaine base (2.5 %). The resulting clear melt was added to the aq. phase with stirring and the resulting anesthetic cream (25 g) had a pH of 6.8.

Rodriguez Bayon AM, Guy RH. **Iontophoresis of nafarelin across human skin in vitro**. Pharm Res

1996 May;13:798-800.
IPA COPYRIGHT: ASHP The iontophoresis of 0.1-1 mg/ml nafarelin acetate across human skin was studied in vitro as a function of the nature of the skin membrane, applied current, donor concentration, and skin metabolism. Iontophoretic delivery of nafarelin was rate limited by the epidermis. Iontophoresis enhanced the transdermal transport of the drug compared to passive penetration. The mechanism of enhanced transport by iontophoresis was electroosmosis. Iontophoretic transport was unexpectedly concentration-dependent, suggesting that the peptide was bound strongly to electronegative skin. Drug metabolism apparently occurred during electrotransport.

Rougier A. **Examples of the use of cell cultures in skin irritancy assessment**. Curr Probl Dermatol 1995;22:214-30.
CBAC COPYRIGHT: CHEM ABS Sequence of events following administration of irritants to skin, in vitro methods for acute skin irritancy testing, cytokine prodn. by activated or damaged keratinocytes and their cellular targets, interleukin 1alpha prodn. by human keratinocytes, three-dimensional cell cultures, episkin treatments, toxicity of phototoxic compds., action of photoprotective compds., surfactants tested on the reconstituted epidermis, cytotoxicity of surfactants in human keratinocyte monolayers and Episkin, UVA and UVB induced cytotoxicity on epidermis, chlorpromazin phototoxicity on epidermis, and the results were discussed.

Shah JC. **Application of kinetic model to in vitro percutaneous permeation of drugs**. Int J Pharm 1996 May 14;133:179-89.
IPA COPYRIGHT: ASHP A kinetic model using inter-compartmental rate constants for permeation of drug across stratum corneum, permeation across viable tissue layer of epidermis, and back transfer of drug from viable tissue layer into the stratum corneum was applied to the in vitro percutaneous permeation process using 4 drugs previously studied across hairless mouse skin and human cadaver skin and the permeation data were fitted to the permeation equation based on the kinetic model to obtain optimized values of rate constants; permeation profiles were also analyzed by the lag time method to estimate steady state flux, lag time, diffusion coefficient, and skin/donor-phase partition coefficient. Permeation data for all drugs, with or without permeation enhancers, were described very well by the model. The rate of drug permeation across stratum corneum was similar to steady state flux.

Shaikh NA, Ademola JI, Maibach HI. **Effects of freezing and azide treatment of in vitro human skin on the flux and metabolism of 8-methoxypsoralen**. Skin Pharmacol 1996;9(4):274-80.
CBAC COPYRIGHT: CHEM ABS The effect of skin storage, prepn., and pretreatment on the permeation and metab. of 8-methoxypsoralen (8-MOP), as a model penetrant, were studied using the flow-through in vitro cell diffusion system. The metabolites and unchanged drug were estd. by TLC. While the permeability of 8-MOP was similar in fresh (445 cm/h) and azide-treated (449 cm/h) skin, decreased permeability was obsd. in frozen skin (406 cm/h). There was a slight increase in the flux of 8-MOP at 24 h when the skin was frozen. There was a moderate relationship between the permeability and flux of 8-MOP through frozen skin. A similar but nonrelated correlation was obsd. between the permeability and flux of 8-MOP through azide-treated-skin samples. Azide and freezing treatments lower the skin barrier properties to the transport of 8-MOP.

Singh SK, Roane DS, Reddy IK, Durrani MJ, Khan MA. **Effect of additives on the diffusion of**

ketoprofen through human skin. Drug Dev Ind Pharm 1996;22(5):471-4.
IPA COPYRIGHT: ASHP The influence of carbomer 934P (Carbopol 934P) and carbomer 940 (Carbopol 940) and the penetration enhancer oleic acid on transdermal permeation of ketoprofen through a full-thickness human skin is reported; permeation parameters such as the flux, permeability coefficient, enhancement ratio, lag time, and partition coefficients are also given for the transdermal ketoprofen patches. Results indicated a maximum flux of ketoprofen from the transdermal patches with carbomer 934P when oleic acid concentration was 35%. The enhancement ratio was 22.8. The maximum flux value for patches made from carbomer 940 was obtained with 10% oleic acid with an enhancement ratio of 34.25. The results indicated that the concentration of oleic acid needed for maximum flux depends on the type of carbomer polymer selected. Further, ketoprofen transdermal drug delivery systems can be fabricated to obtain a zero order release through human skin.

Spielmann H, Liebsch M, Doering B, Moldenhauer F. **First results of the EU/COLIPA validation trial in vitro phototoxicity testing**. In Vitro Toxicol 1996;9(3):325-38.
BIOSIS COPYRIGHT: BIOL ABS. In phase I of a joint prevalidation study six laboratories from COLIPA (the European Cosmetic, Toiletry and Perfumery Association) as well as FRAME (England) and ZEBET (Germany) have evaluated the most promising in vitro methods for phototoxicity testing. Twenty chemicals with known photoirritation properties (11 phototoxins (PT), 5 UV-absorbing non-PT, and 4 non-UV absorbing non-PT) were tested under identical UVA exposure conditions (sun simulation: 5 J/cm2 UVA) in a cytotoxicity assay with 3T3 fibroblasts (endpoint: neutral red uptake, NRU) in all of the laboratories. The chemicals were also tested with in vitro phototoxicity assays established in laboratories of the European cosmetic industry, e.g., photohemolysis and hemoglobin oxidation in red blood cells (RBC), histidine oxidation, a Candida albicans assay, a human lymphocyte assay, a human keratinocyte assay, and, in addition, also in two recently developed commercial assays (SO-LATEX PITM, Skin2TM PI). Development and standardization of the 3T3 NRU phototoxicity test and of the Skin2 phototoxicity assay were carried out at ZEBET's laboratory and are described in detail. During phase I of the EU/COLIPA in vitro phototoxicity study, the 3T3 NRU phototoxicity test, the combined RBC hemolysis and hemoglobin oxidation test, and the Skin2 phototoxicity assay showed the best overall correlation with in vivo data. These assays are currently undergoing a formal validation in phase II of the study, which is conducted in 11 laboratories in Europe and the United States as a blind trial with 30 chemicals carefully selected according to high quality human data.

Squier CA, Kremer M, Wertz PW. **Continuous flow mucosal cells for measuring the in-vitro permeability of small tissue samples**. J Pharm Sci 1997;86(1):82-4.
CBAC COPYRIGHT: CHEM ABS Continuous-flow chambers are described for the measurement of permeability of small tissue samples. The design incorporates a large-capacity donor chamber to permit adequate loading of the applied compd. and a low-vol. (0.3 mL) receiving chamber that ensures rapid removal of penetrant at relatively low (1.5 mL/h or less) pumping rates. Different sized support disks allow tissue biopsies as small as 4 mm in diam. to be utilized. Comparisons of flux and permeability consts. (Kp) for water across oral mucosa indicate that there was no significant difference between values obtained for 10- and 4-mm biopsies. Comparisons of flux and Kp values for porcine oral mucosa and a synthetic membrane between continuous flow and conventional, side-by-side chambers indicated that the latter values were significantly lower, suggesting stasis and inefficient removal of perfusate in

the side-by-side design. The Kp values for water obtained in the continuous-flow chambers with pig skin were similar to those published elsewhere for skin.

Surber C. **Adsorption columns in in vitro percutaneous absorption studies: a novel approach for dermatopharmacokinetics**. Curr Probl Dermatol 1995;22:158-63.
CBAC COPYRIGHT: CHEM ABS In the one-chambered diffusion cell for in vitro percutaneous absorption studies, receptor medium and receptor vol. are key issues. In cases where vol. redn. is not possible, a continuous removal of permeant from the receptor phase by an adequate technique may become difficult. Therefore, a circulating receptor soln. system was developed from which a highly lipophilic model compd., acitretin, was continuously removed by adsorption columns. The use of adsorption columns in a closed circuit of conventional flow-through cell setting significantly improved removal of acitretin from the receptor soln.

Treffel P, Gabard B. **Skin penetration and sun protection factor of ultraviolet filters from two vehicles**. Pharm Res 1996 May;13:770-4.
IPA COPYRIGHT: ASHP The skin penetration of UV filters, 5% oxybenzone, 7.5% octyl methoxycinnamate (2-ethylhexyl 4-methoxycinnamate), and 3% 2-ethylhexyl salicylate, in an emulsion gel or petrolatum (petroleum jelly) was studied in vitro and in 4 healthy subjects (ages 22-31 yr) who received 2 mg/sq cm for 2 min-6 h; sun protection factors (SPFs) were determined 0.5 h after application. In vitro, the emulsion gel generated higher epidermal levels than did petrolatum. Values at 6 h, expressed as percent of applied dose for oxybenzone, octyl methoxycinnamate, and 2-ethylhexyl salicylate were 4, 9, and 7% for the emulsion gel, respectively, and 2, 1, and 2% for petrolatum, respectively. An opposite trend was noticed in deeper skin layers. In vivo, maximum stratum corneum levels for each UV filter occurred at 0.5 h with percentages of applied dose of 50% for the emulsion gel and 15% for petrolatum. SPFs were 14 for the emulsion gel and 5 for petrolatum.

Tsuruta H. **Skin absorption of solvent mixtures. Effect of vehicles on skin absorption of toluene**. Ind Health 1996;34(4):369-78.
BIOSIS COPYRIGHT: BIOL ABS. The skin absorption rates of toluene in various solvent mixtures were investigated in mice. The skin absorption rate of toluene in toluene/methanol mixtures exhibited a parabolic relationship to the mixed ratio. The maximum rate was obtained at a mixed ratio of 50% (V/V). The skin absorption rate of toluene at this point (50%) was about 4.7 times higher than that of pure toluene. The permeability coefficient (Kp) of toluene increased as the mixed ratio of methanol increased. Methanol enhanced skin absorption of toluene. The skin absorption rate of toluene in a toluene/benzene mixture was inversely proportional to the concentration of benzene. The Kp of toluene is kept constant through the mixed ratios of benzene, and benzene does not have an enhancing effect on the skin absorption of toluene contained in the toluene/benzene mixture. We examined the effects of various vehicles on the skin absorption rates of toluene in mixtures containing 50% (v/v) of toluene. Methanol was a good penetration enhancer for toluene, and its effect is similar to the effect of well-known skin penetration enhancers like DMSO, N,N-Dimethylacetamide, and N,N-Dimethylformamide. Therefore, it is necessary to take special precautions against the skin absorption of toluene when handing thinners that contain methanol.

Williams PL, Thompson D, Qiao GL, Monteiro-Riviere N, Riviere JE. **The use of mechanistically**

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

Wu P, Huang Y, Lin H, Tsai Y. **In vitro percutaneous absorption of captopril through excised rabbit skin**. Int J Pharm 1996;143(1):119-23.
CBAC COPYRIGHT: CHEM ABS The permeation characteristics of captopril through excised rabbit skin at various pH values of McIlvaine buffer solns. were investigated. These results indicated that the pH dependency in skin permeability of zwitterionic drug may reflect the permselective property of the skin dependent on the lipophilicity and/or diffusivity of the ionic species. The surfactants were used as penetration enhancers to increase the percutaneous absorption of captopril. These surfactants all showed significant increase (ANOVA, $P<0.05$) in the enhancing effect compared with that of the control group. Among the surfactants, sodium lauryl sulfate showed the greatest effect on the penetration which increased the flux approx. 58.8-fold and the enhancement increased following the increase of surfactant concn.

Xue Y, Zhao J, Weng G, Liu Y, Su M (1). **[Topical penetration of tretinoin in vitro from liposomal gel and its stability]**. Zhongguo Yaoke Daxue Xuebao 1996;27(7):405-7. (Chi)
CBAC COPYRIGHT: CHEM ABS The transdermal penetration of tretinoin in a liposomal gel and in a conventional gel against excised mice skin was compared. The tretinoin amt. of transdermal penetration in the liposomal gel was 1.7 times of that in the gel. While the tretinoin detained in the skin with liposomal from was about 6 times of that with the gel form. The results suggest that liposomes act as a penetration enhancer and, compared with the gel, the liposome formulation improves the local effect of tretinoin in the skin. The stability of tretinoin liposomal gel was studied.

Yokomizo Y, Sagitani H. **Effects of phospholipids on the percutaneous penetration of indomethacin through the dorsal skin of guinea pig in vitro. Part 2. Effects of the hydrophobic group in phospholipids and a comparison with general enhancers**. J Control Release 1996 Oct;42:37-46.

Zhai H, Maibach HI. **Percutaneous penetration (dermatopharmacokinetics) in evaluating barrier creams**. Curr Probl Dermatol 1996;25:193-205.

ECOTOXICITY

Betti C, Nigro M. **The Comet assay for the evaluation of the genetic hazard of pollutants in cetaceans: preliminary results on the genotoxic effects of methyl-mercury on the bottle-nosed dolphin (Tursiops truncatus) lymphocytes in vitro**. Marine Pollut Bull 1996;32(7):545-8.
BIOSIS COPYRIGHT: BIOL ABS. In this study the single cell microgel electrophoresis (Comet assay) was used to evaluate the generic effects of methyl-mercury in dolphin (Tursiops truncatus) lymphocytes in the dose range 1-8 mug ml-1. The results of in vitro exposure showed that methyl-Hg induces DNA single-strand breaks and cytotoxicity in a dose-dependent manner. Dolphin lymphocytes have greater resistance to the genotoxic and cytotoxic effects of methyl-Hg than human and rat cells. The ecological significance of these results are discussed.

Brumley CM, Haritos VS, Ahokas JT, Holdway DA. **Metabolites of chlorinated syringaldehydes in fish bile as biomarkers of exposure to bleached eucalypt pulp effluents**. Ecotoxicol Environ Saf 1996; 33(3):253-60.

Lauth JR, Dyer SD, Belanger SE, Cherry DS. **A novel flow-through method for toxicity assessments using Ceriodaphnia dubia**. Environ Toxicol Water Qual 1996;11(4):335-43.
BIOSIS COPYRIGHT: BIOL ABS. Maintenance of chemical concentrations during toxicity tests is essential to determine the intrinsic toxicity of a material. For compounds that are sorptive, rapidly biodegraded, volatile or chemically labile, static and/or static renewal exposure strategies may result in large fluctuations in exposure concentrations over time, particularly for chronic tests. This article describes the development and adequacy of a novel flow-through chronic toxicity test method for the cladoceran Ceriodaphnia dubia, using a rapidly biodegradable surfactant, dodecyl alkyl sulfate (C12AS) as a model compound. Organisms were exposed to C12AS in polystyrene cups fitted with Nitex mesh on two opposing sides. Cups were placed in either plexiglass or glass flow-through channels. Alkyl sulfate solutions were delivered to the troughs via peristaltic pumps at one end and pumped through the individual test chambers in a linear fashion, exiting at the downstream end. Flow-through tests were conducted with both reconstituted and river water. Control mortality and reproduction met current U.S. Environmental Protection Agency criteria (less than 20% mortality and greater than 15 neonates/ female). Three advantages of this system are (1) the method provides an advance over current Ceriodaphnia dubia chronic test methods for sorptive and labile compounds, (2) it is compact and portable, and (3) it is useful for other small aquatic organism tests.

Mizell M, Romig E, Stegeman J, Smolowitz R, Katayani R. **Zebrafish embryo monitoring of the aquatic environment dose-response synergism revealed in combinations of pollutant chemical mixtures** . Biol Bull1996;191(2):292-4.

BIOSIS COPYRIGHT: BIOL ABS. RRM meeting abstract zebrafish embryo dechorionated aquatic environment monitoring dose-response synergism pollutant chemical mixture benzene embryotoxin toluene development toxicology.

Nacci DE, Cayula S, Jackim E. **Detection of DNA damage in individual cells from marine organisms using the single cell gel assay**. Aquat Toxicol 1996;35(3-4):197-210.
BIOSIS COPYRIGHT: BIOL ABS. The single cell gel (SCG) or comet assay is a simple method by which DNA damage is expressed as relative nuclear 'tail' length of gel-embedded cells following alkaline electrophoresis. While potentially applicable to any cell type, laboratory experiments were conducted to examine the utility of the SCG method for the detection of genotoxicity in cells of marine fish and invertebrates. Selected cells included.

Zahn T, Arnold H, Braunbeck T. **Cytological and biochemical response of R1 cells and isolated hepatocytes from rainbow trout (Oncorhynchus mykiss) to subacute in vitro exposure to disulfoton**. Exp Toxicol Pathol 1996;48(1):47-64.
Ultrastructural, stereological and biochemical alterations in isolated hepatocytes and the permanent fibrocyte-like cell line R1 from rainbow trout (Oncorhynchus mykiss) exposed to 0, 0.2, 2 and 20 mg/l of the phosphorodithioate pesticide disulfoton (Solvirex, O,O-diethyl S-2-ethylthioethyl phosphorodithioate) for up to 5 days were investigated. In both R1 cells and isolated hepatocytes, distinct dose- and time-dependent morphological alterations including diminished amounts of heterochromatin, proliferation of lysosomal elements, dilation and vesiculation of endoplasmic reticulum cisternae, induction of concentric membrane whorls and an increased amount of lipid droplets could be detected at concentrations of > or = 2 mg/l (R1 cells) and > or = 0.2 mg/l disulfoton (hepatocytes). Additional effects in isolated hepatocytes comprised marginalization of heterochromatin, myelin-like structures attached to mitochondrial membranes, formation of ring-shaped mitochondria, proliferation of smooth endoplasmic reticulum, reduction of rough endoplasmic reticulum, induction of ring-shaped Golgi cisternae, glycogen depletion and occurrence of glycogenosomes. Structural changes in isolated hepatocytes could be correlated to suppression of lactate dehydrogenase, glucose-6-phosphate dehydrogenase, alanine aminotransferase, malic enzyme, esterase as well as glutathione S-transferase, but to a stimulation of 7-ethoxycoumarin-O-deethylase and the rate of lipid peroxidation at concentrations > or = 0.01 mg/l disulfoton. Comparison with data from in vivo experiments with rainbow trout indicate the suitability of in vitro techniques for the evaluation of the toxicological potential of a wide range of ecotoxicologically relevant substances.

GENOTOXICITY AND MUTAGENESIS

Organ variation in the mutagenicity of ethylnitrosourea in Muta mouse: results of the collaborative study on the transgenic mutation assay by JEMS/MMS. The Collaborative Study Group for the Transgenic Mouse Mutation Assay Mammalian Mutagenesis Study Group of the Environmental Mutagen Society of Japan. Environ Mol Mutagen 1996;28(4):363-75.
A collaborative study of the transgenic mouse mutation assay was performed by a subgroup of the Mammalian Mutagenesis Study Group (MMS), which is a suborganization of the Environmental

Mutagen Society of Japan (JEMS). Twenty-six laboratories participated in this collaboration, and ethylnitrosourea (ENU) mutagenesis was studied in eight organs of lacZ transgenic mice (Muta Mouse)-liver, spleen, bone marrow, brain, lung, kidney, urinary bladder, and heart. Mice were treated by a single intraperitoneal (ip) injection of 150 mg/kg ENU, and the lacZ mutant frequency (MF) was analyzed by positive selection after 3- and 14-day expression times. The MF in the control group was similar for all organs, approximately 40-60 x 10(-6). ENU increased MF in all organs except the brain with the highest values (more than 10 times the control value) observed in bone marrow on days 3 and 14, and in spleen on day 14. The MF in urinary bladder increased over 400 x 10(-6) and MFs in liver and lung were more than 150 x 10(-6) on day 14, although no increases were apparent in these organs on day 3. Approximately a doubling of control values was observed in kidney and heart but these were less than 100 x 10(-6). These results demonstrated that ENU induces organ specific mutagenesis with specific expression periods.

Transgenic animals in mutation research. Satellite conference to the 1996 meeting of the Environmental Mutagen Society. Sidney, British Columbia, Canada, March 20-23, 1996. Environ Mol Mutagen 1996;28(4):295-489.

Aminov RI, Nagamine T, Ogata K, Sugiura M, Tajima K, Benno Y. **Cloning, sequencing and complementation analysis of the recA gene from Prevotella ruminicola**. FEMS Microbiol Lett 1996;144(1):53-9.
Degenerate PCR primers based on conserved RecA protein regions were used to amplify a portion of recE from Prevotella ruminicola strain 23, which was used as a probe to isolate the full-length recA gene from the P. ruminicola genomic library. The P. ruminicola recA gene encoded a protein of 340 amino acids with a molecular mass of 36.81 kDa, P. ruminicola RecA was highly similar to other RecA proteins and most closely resembled that of Bacteroides fragilis (75% identity). It alleviated the methyl methanesulfonate and mitomycin C sensitivities of Escherichia coli recA mutants, but did not restore the resistance to UV-light irradiation. Mitomycin C treatment of otherwise isogenic E. coli strains showed a higher level of prophage induction in a recA harboring lysogen.

Anderson D, Blowers SD, Marrs TC, Rice P. **An in vitro and an in vivo unscheduled DNA synthesis assay with a zinc oxide/hexachloroethane (Zn/HCE) smoke**. Hum Exp Toxicol 1996;15(1):38-44.
In an effort to clarify the mutagenic effects of smoke from a mixture of zinc-oxide (1314132) and hexachloroethane (67721) (Zn/HCE) following reports of contrasting results in the Ames test and the bone marrow micronucleus assay, the ability of Zn/HCE smoke to induce unscheduled DNA synthesis (UDS) in rat hepatocytes in-vivo and in-vitro was examined. For in-vivo studies, male Fischer-344-rats were exposed to the Zn/HCE smoke at different concentrations for 1 hour. Sixteen hours later, rats were terminally anaesthetized, and hepatocytes were isolated for measurement of UDS. In-vivo, Zn/HCE in doses of approximately 20 and 56 micrograms/liter induced nonsignificant dose related increases in UDS. For In-vitro studies hepatocytes from rats were incubated with medium exposed to Zn/HCE smoke. In in-vitro studies, exposure to levels between 0.12 and 3,360.0 nanograms zinc (Zn) per milliliter did not significantly increase the percentage of cells in scheduled or unscheduled DNA synthesis; however, exposures to 1,900 or 5,700 nanograms Zn/milliliter were toxic, reducing the apparent cell size. The authors conclude that Zn/HCE smoke does not appear to be genotoxic.

Anderson D, Dhawan A, Yu TW, Plewa MJ. **An investigation of bone marrow and testicular cells in vivo using the comet assay**. Mutat Res 1996;370(3-4):159-74.
The effects of the mutagens, cyclophosphamide (CP), ethyl methanesulphonate (EMS), bleomycin (BLM) and the testicular toxin ethylene glycol monomethyl ether (EGME) in bone marrow and testicular cells have been compared in the alkaline COMET assay. Sprague-Dawley rats were administered by gavage with 50, 100 and 150 mg/kg body weight (bw) of CP; 100, 200 and 300 mg/kg bw EMS; 50, 100 and 150 mg/kg bw BLM and 500, 1000 and 1500 mg/kg bw EGME. Effects were examined at week 2 after treatment for CP, EMS BLM and EGME and at weeks 5 and 6 for EGME. Bone marrow cells were removed and separated by aspiration of the femur and testicular cells by decapsulation of the testis, treating with collagenase followed by trypsin. Various statistical methods were used to analyse the data. For CP there was an increase in damage above control values for bone marrow at 50 mg/kg bw which decreased at 100 mg/kg bw, and there was mortality of the animals at 150 mg/kg bw. A similar response was found in the testicular cells. For EMS and BLM, there were only occasional slight increases in damage in bone marrow and testicular cells. Two studies were conducted with EGME. In the first, where effects were examined at week 2 after treatment, there was an increase in damage in bone marrow cells, but a larger response was observed in testicular cells. In the second study where effects were examined at weeks 5 and 6 after treatment, bone marrow and testicular cells were not affected. The overall results showed that damage persisted for 2 weeks after treatment with CP and EGME but not in weeks 5 and 6 for EGME. Various statistical methods were used to analyse the data. Statistically significant responses were produced after treatment with CP and EGME and were dose-related for EGME, but after treatment with EMS and BLM statistical increases were sporadic. These results suggest that the assay is useful for measuring DNA damage and its persistence, and for comparing the sensitivity of different target organs in vivo.

Atoiants AL, Pogosian VS, Agadzhanian EA, Kasparova IP, Arutiunian RM. **[An evaluation of the mutagenic activity of the air pollutants in the area of a plant for the manufacture of synthetic rubber by using Tradescantia (clone 02) as the test object]**. Tsitol Genet 1996;30(3):26-31. (Rus)
The mutagenic effects of atmospheric pollutants of synthetic rubber works on the frequency of point mutations in somatic cells of stamen hairs (CSH) of Tradescantia (clone 02) was studied under the condition of functioning or nonoperating shops. It was shown that temporary stopping of shops led to a decrease in the led to a decrease in the number of recessive mutations in CSN to the level 1.5 times higher than the control one. It increased during shop functioning to 0.21%. The cells moving off from the source of pollution led to a decrease in mutation frequency. The changes in the frequency of genetically indefinite mutations and morphological traits were also registered.

Baier H, Klostermann S, Trowe T, Karlstrom RO, Nusslein-Volhard C, Bonhoeffer F. **Genetic dissection of the retinotectal projection**. Development 1996;123:415-25.
A systematic search for mutations affecting the retinotectal projection in zebrafish larvae was performed, as part of the large-scale Tubingen screen for homozygous diploid mutants in embryonic development. 2,746 inbred lines (F2 families) from males mutagenized with ethylnitroso urea were screened. In wild-type larvae, developing retinal axons travel along a stereotyped route to the contralateral optic tectum. Here, their terminals form a highly ordered retinotopic map. To detect deviations from this pattern, an axon tracing assay was developed that permits screening of large

numbers of mutagenized fish. Two fluorescent tracer dyes (DiI and DiO) were injected at opposite poles of the eyes of day-5 aldehyde-fixed larvae. 12 hours later, retinal axons were labelled over their entire length, and could be observed through the intact skin. The assay procedure (aldehyde fixation, mounting, injection of dyes, microscopic analysis) took about 1 minute per fish. In total, 125,000 individual fish larvae were processed. During the screen, 114 mutations in approx. 35 genes were discovered. For the mutants subjected to complementation testing, the number of alleles per locus ranges from 1 to 15. The mutations affect distinct steps in the retinotectal pathway, from pathfinding between eye and tectum to map formation along the dorsal-ventral and the anterior-posterior axis of the tectum. Mutations that disturb axon pathfinding to the tectum for the most part do not disrupt retinotopic mapping, and vice versa. The majority of the mutants display associated defects in other tissues and die before day 10. These mutants provide new tools for studying the formation of neuronal maps. The results of this screen show that a large-scale genetic approach can be applied to relatively late and circumscribed developmental processes in the vertebrate brain.

Basaran AA, Yu TW, Plewa MJ, Anderson D. **An investigation of some Turkish herbal medicines in Salmonella typhimurium and in the COMET assay in human lymphocytes**. Teratog Carcinog Mutagen 1996;16(2):125-38.
Medicinal plants play a major role in the life of Turkish people and of late medicinal plant usage has increased in many countries. Green plants in general contain mutagenic and carcinogenic substances, but there is little information about the biological activities of herbal medicine. In the present study, therefore, various Turkish medicinal herbs were investigated for their genotoxic potential in the Salmonella typhimurium microsomal activation assay and the alkaline single cell gel electrophoresis (COMET) assay. Extracts from these medicinal herbs and some fractions of these extracts were examined. The species investigated were Arctium minus, Ecballium elatterium, Momordica charantia, Plantago major, Urtica dioica, Viscum album, Salvia triloba, Euphorbia rigida, Stachys lavandulifolia, Acteoside, Abies nordmannia. They are used for various immune disorders and are applied either topically or taken orally as a herbal tea. Of the 19 samples of the extracts and fractions investigated, none produced a positive response in strains TA98 and TA100 with or without metabolic activation, but all produced an increase above negative control values in the COMET assay. Some extracts were investigated further and produced dose-related increases. In the case of Urtica and Euphorbia species, where two fractions from these plants were examined, one fraction produced a greater response than the other. It is suggested that the lesser response of the fractions might be due to less DNA strand-breaking agents in the fractions or they may have antigenotoxic properties. The breaks that are detected in the COMET assay could be alkali-labile AP-sites and intermediates.

Brooks AL, McDonald KE, Mitchell C, Culp DS, Lloyd A, Johnson NF, Kitchin RM. **The combined genotoxic effects of radiation and occupational pollutants**. Appl Occup Environ Hygiene 1996;11 (4):410-6.
A study was conducted to determine how short term tests can be used as first screens to evaluate the potential interaction between damage induced by radiation and chemicals present in the work environment. The materials chosen for study were present in the work environment in amounts and forms that represent a potential for worker exposure. Cell killing and the induction of chromosome damage in tissue culture following exposure to the metal beryllium (7440417), the fiber silicon-carbide

(409212), and the organic solvents hexone (108101) and tributyl-phosphate (126738) were studied. Synergistic interactions were observed.

Bruner LH, Carr GJ, Chamberlain M, Curren RD. **Validation of alternative methods for toxicity testing**. Toxicol In Vitro 1996;10(4):479-501.
BIOSIS COPYRIGHT: BIOL ABS. Many studies have been conducted in order to assess the validity of alternative methods as replacements for in vivo toxicity tests. The purpose of this review is to build on what has been learned in the course of this work by presenting a practical process that can be used to conduct future validation programmes. The important role of a clearly stated prediction model, which defines how to use the results from an alternative method to predict an in vivo toxicity endpoint, has been introduced. Computer simulations have been used to demonstrate that data-based guidance can be developed to assist in judging the performance of alternative methods assessed in a validation study. Additionally, statistical procedures have been used in order to provide guidance on choosing the appropriate number of reference test substances and number of participating laboratories to include in a validation study. The validation of alternative methods for eye irritation testing is used as a specific example to illustrate important concepts. Although the focus of the discussion is on the validation of alternative methods intended to replace current in vivo tests, the procedures can be used to assess the performance of alternative methods intended for other uses. This review will be particularly useful to those who require a practical guide for conducting a validation study and to those who must assess the results of such programmes.

Buenger J, Stork J, Stalder K. **Cyto- and genotoxic effects of coordination complexes of platinum, palladium and rhodium in vitro**. Int Arch Occup Environ Health 1996;69(1):33-8.
BIOSIS COPYRIGHT: BIOL ABS. The growing industrial use of platinum group elements as catalysts, especially in automobile exhaust detoxification (trimetal catalytic converters), is causing increasing occupational and environmental pollution. The cytotoxic and mutagenic properties of industrially used coordination complexes of platinum, palladium and rhodium were investigated using the neutral red cytotoxicity assay on two established cell lines and the Salmonella typhimurium/microsome test system (Ames test). Cytotoxic effects of the platinum complexes, measured as ED50, occurred at test concentrations of 0.2 mM. The analogous palladium salts tested were 3 times less toxic with ED50 being 0.6 mM, while the rhodium salts proved to be 30 times less toxic (ED50 = 6 mM). Levels of toxicity of the different complexes of a particular metal did not differ significantly from each other, which indicates that the metal itself is responsible for the toxic effects. In the Ames test, the spontaneous mutation rates increased by factors of 3 to 20 when the four tester strains were exposed to the platinum complexes. The analogous rhodium compounds proved to be considerably less mutagenic, and palladium demonstrated no mutagenic potential. As all of the four tester strains contain different mutations, the mutagenic potential of platinum and rhodium complexes appears to be based on a variety of mechanisms that damage DNA. From these in vitro experiments, it can be concluded that water-soluble complex salts of rhodium are less.

Butler WH, Gabriel KL, Preiss FJ, Osimitz TG. **Lack of genotoxicity of piperonyl butoxide**. Mutat Res 1996;371(3-4):249-58.
The genotoxicity of piperonyl butoxide has been investigated in bacterial mutation assays using tester strains TA98, TA100, TA1535, TA1537 and TA1538. The assays were conducted both with and without

metabolic activation. Piperonyl butoxide was tested for mutation with and without metabolic activation in the CHO/HGPRT assay. Chromosomal aberrations were investigated also using Chinese hamster ovary (CHO) cells and effects on DNA were evaluated by in vitro unscheduled DNA synthesis (UDS) test using rat liver primary cell cultures. Piperonyl butoxide was not shown to be genotoxic in any assay system. The data presented supports the view that the liver tumors observed in rodents at dose levels above the maximally tolerated dose (MTD) result from a secondary non-genotoxic mechanism.

Candido EP, Jones D. **Transgenic Caenorhabditis elegans strains as biosensors**. Trends Biotechnol 1996;14(4):125-9.
Toxicity bioassays rely largely on lethality measurements. Such assays are generally lengthy and expensive, and provide little information on mechanisms of toxicity. A desire to understand the mechanisms by which cells respond to physical and chemical stresses has led to interest in measuring stress proteins as toxicological endpoints. Transgenic strains of the nematode Caenorhabditis elegans that carry a reporter enzyme under control of a stress-inducible promoter have been created. The reporter is easily quantified in intact nematodes, and it responds to a wide range of chemical stressors. Therefore, transgenic C. elegans can provide the basis for a wide range of quick, simple and informative bioassays.

Chiang LW. **Saturation mutagenesis by mutagenic oligonucleotide-directed PCR amplification (Mod-PCR)**. Methods Mol Biol 1996;57:311-21.

Choi YJ, Kim CJ, Ji GE. **A partially purified beta-glucosidase from Bifidobacterium adolescentis converts cycasin to a mutagenic compound**. Lett Appl Microbiol 1996;22(2):145-8. beta-Glucosidase was extracted from sonicated Bifidobacterium adolescentis Int-57 and partially purified by Sepharose CL-6B gel-filtration and DEAE-cellulose ion-exchange chromatography. The partially purified enzyme was confirmed to convert cycasin to a mutagen in the Ames and SOS chromotests. beta-Glucosidase negative strains were unable to activate cycasin mutagenically.

Chopkiewicz B, Ejchart A, Marczewska J. **Studies on the mechanism of hydralazine induced mutagenicity and genotoxicity**. Acta Pol Pharm 1995;52(3):219-22.
The mutagenicity (Ames test) and genotoxicity (SOS Chromotest) of hydralazine were studied. Hydralazine was found to be genotoxic to E.coli PQ37. In experiments with E.coli MD332 it was genotoxic in responsive temperature (30 degrees C) but not genotoxic in responsive temperature (30 degrees C) but not genotoxic in non-responsive temperature (42 degrees C). Hydralazine was mutagenic to S.typhimurium TA100 and TA104 but not mutagenic to TA102. Different active oxygen species scavengers did not influence the genotoxicity and mutagenicity of hydralazine.

Cirrincione G, Almerico AM, Grimaudo S, Diana P, Mingoia F, Barraja P, Misuraca F. **3-Diazopyrroles. Part 6. Mutagenic activity of 3-diazopyrroles in Streptomyces coelicolor A3(2) during various phases of growth**. Farmaco 1996;51(1):49-52.
3-Diazopyrroles, a class of compounds particularly interesting from a chemical and biological point of view, were assayed for their ability to induce gene mutations employing back mutation (his+ reversion) test in the philamentous bacterium Streptomyces coelicolor at various time during life cycle. Our results suggest that in evaluating the mutagenicity and toxicity of chemicals in Streptomyces system it is

important to consider factors such as growth phase. Furthermore in this series of diazopyrroles a relationship between toxicity, mutagenicity and chemical structure was found. The observed mutagenic activity can be the molecular basis for the appearance of antitumor activity.

Clouter A, Houghton CE, Bowskill CA, Hoskins JA, Brown RC. **An in vitro-invivo study into the short term effects of exposure to mineral fibres**. Exp Toxicol Pathol 1996;48(6):484-6.
BIOSIS COPYRIGHT: BIOL ABS. RRM Research article rat toxicology mineral fiber calcium sulfate crocidolite toxicodynamics.

De Rubertis F, Kadosh D, Henchoz S, Pauli D, Reuter G, Struhl K, Spierer P. **The histone deacetylase RPD3 counteracts genomic silencing in Drosophila and yeast**. Nature 1996;384(6609):589-91.
Both position-effect variegation (PEV) in Drosophila and telomeric position-effect in yeast (TPE) result from the mosaic inactivation of genes relocated next to a block of centromeric heterochromatin or next to telomeres. In many aspects, these phenomena are analogous to other epigenetic silencing mechanisms, such as the control of homeotic gene clusters, X-chromosome inactivation and imprinting in mammals, and mating-type control in yeast. Dominant mutations that suppress or enhance PEV are thought to encode either chromatin proteins or factors that directly affect chromatin structure. We have identified an insertional mutation in Drosophila that enhances PEV and reduces transcription of the gene in the eye-antenna imaginal disc. The gene corresponds to that encoding the transcriptional regulator RPD3 in yeast, and to a human histone deacetylase. In yeast, RRD3-deletion strains show enhanced TPE, suggesting a conserved role of the histone deacetylase RPD3 in counteracting genomic silencing. This function of RPD3, which is in contrast to the general correlation between histone acetylation and increased transcription, might be due to a specialized chromatin structure at silenced loci.

Deleve LD. **Dinitrochlorobenzene is genotoxic by sister chromatid exchange in human skin fibroblasts**. Mutat Res 1996;371(1-2):105-8.
Dinitrochlorobenzene (DNCB) is clinically efficacious in the therapy of alopecia areata, but its use was limited when it was found to be mutagenic in the Ames test. However, there has been renewed interest in the immunomodulatory benefits of topically applied dinitrochlorobenzene in patients with human immunodeficiency virus and systemic lupus erythematosus. The current study examines the genotoxicity of dinitrochlorobenzene in human skin fibroblasts using sister chromatid exchange.

Demarini DM, Shelton ML, Bell DA. **Mutation spectra of chemical fractions of a complex mixture: role of nitroarenes in the mutagenic specificity of municipal waste incinerator emissions**. Mutat Res 1996; 349(1):1-20.
Effluent samples from a municipal waste incinerator were extracted with dichloromethane (DCM), and the organic extract was fractionated by a nonaqueous ion exchange separation procedure. The fractions and the whole extract were evaluated for mutagenicity in a microsuspension Salmonella assay, with or without rat liver S9 mix. Molecular analysis of about 3,000 revertants via colony probe hybridization and DNA sequence analysis was conducted. The DCM and methanol (MeOH) fractions exhibited higher mutagenic potencies than did the whole extract and the carbon-dioxide (CO2)/MeOH, 2% trifluoroacetic acid (TFA)/MeOH, and 10% TFA/MeOH fractions. Mutagenic potencies in Salmonella strains deficient in nitroreductase (TA-98NR) and transacetylase (TA-98/1,8-DNP6) indicated that nitroarenes accounted for the greatest amount of the direct acting mutagenicity. At least 50% of the direct acting mutagenicity

was from nitroarenes that eluted in the neutral/base fraction. In TA-98, these nitroarenes induced only a hotspot two base deletion. Most complex frameshifts induced by the whole extract were induced by nitroarenes activated by transacetylation and which were found in the polar neutral fraction. The authors conclude that nitroarenes are a significant mutagenic component of municipal waste incinerator emissions, and that certain classes of chemicals account for the mutagenicity of complex chemical mixtures.

Dennog C, Hartmann A, Frey G, Speit G. **Detection of DNA damage after hyperbaric oxygen (HBO) therapy**. Mutagenesis 1996;11(6):605-9.
CBAC COPYRIGHT: CHEM ABS We investigated the DNA-damaging effect of hyperbaric oxygen (HBO) with the alk. version of the single cell gel test (Comet assay). Oxidative DNA base modifications were detd. by converting oxidized DNA bases to strand breaks using bacterial formamidopyrimidine-DNA glycosylase (FPG), a DNA repair enzyme, which specifically nicks DNA at sites of 8-oxo-guanines and formamidopyrimidines. HBO treatment under therapeutic conditions clearly and reproducibly induced DNA.

Duthie SJ, Collins AR. **The influence of cell growth, detoxifying enzymes and DNA repair on hydrogen peroxide-mediated DNA damage (measured using the comet assay) in human cells**. Free Radical Biol Med 1997;22(4):717-24.
CBAC COPYRIGHT: CHEM ABS The authors have used the comet assay to investigate the influence of growth state, xenobiotic detoxifying enzymes, and DNA repair processes on the response of cultured human cells to oxidative damage. HepG2 and Caco-2 cells are differentiated liver and colon cell lines, resp. HeLa and GM1899A cells are relatively unspecialized epithelial and lymphoblastoid cells. Substrate-dependent cells showed a cyclical fluctuation of glutathione (GSH) with respect to growth. Enzyme activities (glutathione reductase, glutathione peroxidase, and catalase) varied considerably between cell types and changed with cell growth state. Hydrogen peroxide induced more DNA damage in actively dividing cells than in confluent cultures. Sensitivity to oxidative injury did not correlate with detoxifying enzyme activity. Rather, differences in susceptibility between cells could be correlated with differences in DNA repair capacity. This study highlights the need to standardize exptl. conditions if the comet assay is to be employed in the study of genotoxicity.

Duverger-Van Bogaert M, Dierickx PJ, Crutzen MC. **Mutagenic activation of aromatic amines by a human hepatoma cell (Hep G2) supernatant tested by means of Salmonella typhimurium strains with different acetyltransferase activities**. Mutat Res 1995;335(3):219-27.
A study was undertaken to assess the facility of a human hepatoma cell (Hep-G2) activation system and several Salmonella-typhimurium tester strains in mutagenic assays of aromatic amines. Supernatant was collected from Hep-G2 cell cultures and used to activate the aromatic amines benzidine (92875), 2-aminofluorene (153786) (2-AF), and 2-acetylaminofluorene (53963) (2-AAF). S-typhimurium tester strains TA-98, YG1024, DJ400, and DJ460 were used to measure levels of Hep-G2 activation in the presence and absence of benz(a)anthracene (56553) (BA). These results were compared with those taken from rat liver S9 studies of male Wistar-rats given single doses of 500mg/kg of Aroclor-1254 and their controls. Strains YG1024, DJ400, and DJ460 showed signs of 2-AF and 2-AAF activation. Results were similar for Hep-G2 and rat liver S9 trials, although 2-AF was better activated using rat liver S9. The authors conclude that aromatic amines can be activated into mutagens by Hep-G2 supernatant, that this

process is increased in the presence of BA, and that in the rat liver S9 metabolic activation system, acetyltransferase activity limits mutagenic activity.

Farningham DA, Damment SJ, West C. **Report of the meeting on 'Animal Welfare in Regulatory Toxicology' under the auspices of the British Toxicology Society, 27 April 1995**. Hum Exp Toxicol 1996;15(5):452-4.

Flowers L, Bleczinski WF, Burczynski ME, Harvey RG, Penning TM. **Disposition and biological activity of benzo[a]pyrene-7,8-dione. A genotoxic metabolite generated by dihydrodiol dehydrogenase**. Biochemistry 1996;35(42):13664-72.
A novel pathway of polycyclic aromatic hydrocarbon metabolism involves the oxidation of non-K-region trans-dihydrodiols to yield o-quinones, a reaction catalyzed by dihydrodiol dehydrogenase (DD). We have recently shown that in isolated rat hepatocytes (+/-)-trans-7,8-dihydroxy-7,8-dihydrobenzo-[a] pyrene (BP-diol) was oxidized by this route to yield benzo [a] pyrene-7,8-dione (BPQ). We now report the disposition of BPQ and its mutagenic and genotoxic properties. Using [3H]BPQ it was found that 30% of the radioactivity was sequestered by rat hepatocytes into the cell pellet. Isolation of hepatocyte DNA provided evidence for a low level of covalent incorporation of BPQ into DNA (30 +/- 17 adducts/ 10(6) base pairs). Examination of the hepatocellular DNA by agarose gel electrophoresis following treatment with BPQ indicated that extensive fragmentation had occurred. DNA fragmentation was also observed when hepatocytes were treated with BP-diol and this effect was attenuated by indomethacin, a DD inhibitor. Hepatocytes treated with either BP-diol or BPQ were found to produce large quantities of superoxide anion radical (O2.-). The amount of O2.- generated by BP-diol was blocked by DD inhibitors. These data suggest that by diverting BP-diol to BPQ reactive oxygen species (ROS) were generated which caused DNA fragmentation. The ability of BPQ to cause DNA strand scission was further studied using supercoiled phi X174 DNA. It was found that BPQ caused concentration-dependent (0.05-10 microM) strand scission in the presence of 1 mM NADPH (which promoted redox-cycling) provided CuCl2 (10 microM) was present. Complete destruction of the DNA was observed using 10 microM BPQ. This strand scission was prevented by catalase and hydroxyl radical scavengers but not by superoxide dismutase. These data indicate that ROS were responsible for the destruction of the DNA. Using 20 microM (+/-)-anti-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo [a]pyrene [(+/-)-anti-BPDE] only single nicks in the DNA were observed indicating that BPQ was the more potent chemical nuclease. BPQ was also found to be a direct-acting mutagen in the Ames test using Salmonella typhimurium tester strains TA97a, TA98, TA100, TA102, and TA104, but was 10-5500-fold less efficient as a mutagen than (+/-)-anti-BPDE. Our data indicate that DD suppresses the mutagenicity of (+/-)-anti-BPDE by producing BPQ, but in doing so a potent chemical nuclease is produced which causes extensive DNA fragmentation via the generation of ROS.

Fritz A, Rozowski M, Walker C, Westerfield M. **Identification of selected gamma-ray induced deficiencies in zebrafish using multiplex polymerase chain reaction**. Genetics 1996;144(4):1735-45.
BIOSIS COPYRIGHT: BIOL ABS. The ease with which mutations can be generated in zebrafish makes this vertebrate an important resource for developmental genetics and genome studies. We have developed a PCR-based screening method that allows the efficient identification of gamma-ray induced deficiencies targeted to selected sequences. We describe three mutants characteristic of our findings and

show that these mutations include deletions and translocations that can affect as much as 1% of the genome. These deficiencies provide a basis for analyzing the functions of cloned zebrafish genes using noncomplementation screens for point mutations induced by high-efficiency chemical mutagenesis.

Gaiano N, Amsterdam A, Kawakami K, Allende M, Becker T, Hopkins N. **Insertional mutagenesis and rapid cloning of essential genes in zebrafish**. Nature 1996;383(6603):829-32.
Large-scale chemical mutagenesis screens in zebrafish have led to the isolation of thousands of lethal mutations in genes that are essential for embryonic development. However, the cloning of these mutated genes is difficult at present as it requires positional cloning methods. In Drosophila, chemical mutagenesis screens were complemented with P-element insertional mutagenesis which facilitated the cloning of many genes that had been identified by chemical lesions. To facilitate the cloning of vertebrate genes that are important during embryogenesis, we have developed an insertional mutagenesis strategy in zebrafish using a retroviral vector. Here, in a pilot screen of 217 proviral insertions, we obtained three insertional mutants with embryonic lethal phenotypes, and identified two of the disrupted genes. One of these, no arches, is essential for normal pharyngeal arch development, and is homologous to the recently characterized Drosophila zinc-finger gene, clipper, which encodes a novel type of ribonuclease. As it is easy to generate tens to hundreds of thousands of proviral transgenes in zebrafish, it should now be possible to use this screening method to mutate and then rapidly clone a large number of genes affecting vertebrate developmental and cellular processes.

Galli A, Schiestl RH. **Effects of Salmonella assay negative and positive carcinogens on intrachromosomal recombination in G1-arrested yeast cells**. Mutat Res 1996;370(3-4):209-21.
A wide variety of carcinogens including Ames assay (Salmonella) positive as well as Salmonella-negative carcinogens induce intrachromosomal recombination (DEL recombination) in strain RS112 of Saccharomyces cerevisiae. It has been previously shown that the Salmonella-positive carcinogens ethyl methanesulfonate (EMS), methyl methanesulfonate (MMS) and 4-nitroquinoline-N-oxide (4-NQO) and the Salmonella-negative carcinogens safrole, benzene, thiourea, carbon tetrachloride and urethane induce DEL recombination in G2-arrested yeast cells. DEL recombination is preferentially induced by safrole, benzene and carbon tetrachloride in G2-arrested cells which might be explained by preferential induction of unequal sister chromatid recombination leading to deletions. To test this, cells of strain RS112 were arrested in the G1 phase of the cell cycle, exposed to these carcinogens and the frequencies of DEL and interchromosomal recombination (ICR) were determined. EMS, MMS and 4-NQO induced DEL recombination and ICR in G1-arrested cells with a linear dose-response curve. In contrast, the Salmonella-negative carcinogens safrole, benzene, carbon tetrachloride, thiourea and urethane induced DEL recombination and ICR with a threshold below which no significant increase was seen and only at already cytotoxic doses. EMS, MMS and 4-NQO were more recombinagenic in previous experiments with growing cells than in G1-arrested cells. On the other hand, safrole, benzene and carbon tetrachloride were more recombinagenic in G1-arrested than in growing cells. Thus, inducibility of DEL recombination in G1-arrested cells parallels inducibility in G2-arrested cells making it less likely that sister chromatid recombination events might be involved. These data are discussed in terms of the mechanism of induced DEL recombination and the possible biological activities of these carcinogens.

George SE, Kohan MJ, Warren SH. **Hepatic DNA adducts and production of mutagenic urine in 2,6-dinitrotoluene-treated B6C3F1 male mice**. Cancer Lett 1996;102(1/2):107-11.

Induction of hepatic DNA adducts and production of mutagenic urine by 2,6-dinitrotoluene (606202) (26DNT) were studied in mice. Male B6C3F1-mice were gavaged daily with 0 or 5mg/kg 26DNT for 3 days. Urine samples were collected daily and tested for mutagenicity in the Ames/Salmonella assay using strain TA-98 as the tester strain. Mice were killed after the last 26DNT dose and the livers were removed. The DNA was extracted and analyzed for adducts using the phosphorus-32 post labeling assay. The data were compared with results obtained in previous studies conducted in CD-1-mice and Fischer-344-rats. Mutagenic 26DNT metabolites were excreted by B6C3F1-mice. The level of mutagenicity was 469 revertants per milliliter (ml) urine was comparable to that obtained in CD-1-mice and Fischer-344-rats, 624 and 490revertants/ml, respectively. Two distinct 26DNT/DNA adducts were detected in B6C3F1-mice, the concentrations being 0.8 and 0.6 relative adduct levels per 10(8) nucleotides (RAL/10(8)). By contrast, four adducts having concentrations of 4.6, 5.7, 4.5, and 2.1RAL/10(8) were detected in the livers of 26DNT treated Fischer-344-rats. The authors conclude that oral treatment of B6C3F1-mice with 26DNT results in the production of mutagenic urine and formation of hepatic DNA adducts. The pattern of DNA adduction was different from that of Fischer-344-rats. This finding supports the results of other studies which have reported that 26DNT metabolism is species and strain dependent.

Gershenzon SM. **[The selectivity of the mutagenic action of DNA and other polynucleotides]**. Zh Obshch Biol 1996;57(6):661-83. (Rus)
A paper summarises the results of author's and his collaborators studies on the mutagenic action of DNA and other natural and synthetic polynucleotides. These results were published with rare exception in Russian and Ukrainian scientific journals. Analogous results obtained by other authors of several countries are also discussed. It was proved that unlike conventional physical and chemical mutagens, polynucleotides selectively induce visible and lethal mutations preferentially in certain genes, different in case of treatment with different polynucleotides. Polynucleotides often induce mutation in several non-allelic genes in a single germ cell of the host (multimutational effect). Polynucleotides do not induce gross chromosome aberration.

Gocke E. **Review of the genotoxic properties of chlorpromazine and related phenothiazines**. Mutat Res 1996;366(1):9-21.
Chlorpromazine and related phenothiazine drugs have been used in human and veterinary medications for more than 40 years, predominantly as psychotropic agents. Genotoxicity reports are in many cases of relatively antiquated test design. Overall there appears to be no genotoxic activity associated with these drugs when tested under standard conditions. Limited evidence for the potential to form mutagenic nitrosation products and some indication for the ability to modulate the genotoxic action of various mutagens have been presented in the literature. UV irradiation of chlorpromazine and other chlorinated derivatives produces reactive free radicals which possess DNA damaging properties. Induction of gene mutation and chromosomal aberrations have been observed in appropriately designed photomutagenesis experiments. Enhancement but also reduction of UV induced skin tumour formation by chlorpromazine have been found. The decisive factor for the discrepant actions has not been recognized. It is clearly advisable to avoid extensive UV exposure during therapy with these drugs.

Gotlib VIA, Serebrianyi AM, Chernikova SB, Kudriashova OV, Pelevina II. **[A comparison of the patterns of delayed cell death after exposure to genotoxic agents]**. Tsitologiia 1996;38(9):974-82.

(Rus)
A lot of data have been provided on different types of cells showing that ionizing radiation induces a hereditable genome instability, which may lead to mutations chromosome aberrations and cell death. In this paper we studied delayed death, proliferative activity, sensitivity to genotoxic agents to progeny of HeLa and LL cells following treatment with ionizing radiation, cis-platinum, methylhydroxurea which induce different types of lesions with different rate of repair. The rate of death of the progeny, dynamics of the clonogen ability recovery, growth rate recovery after the treatment with genotoxic agents are different. We have supposed that the delayed cell death may be associated with different types of hereditable lesions.

Govorun RD, Koshlan' IV, Krasavin EA, Shmakova NL. **[The mutagenic action of gamma radiation on Chinese hamster cells. The cytogenetic characteristics of the mutants at the HPRT locus]**. Radiats Biol Radioecol 1996;36(6):852-9. (Rus)
The peculiarities of mutation induction and cytogenetic characteristics of spontaneous and radiation-induced HPRT mutant clones have been studied. The linear-quadratic dependence of the mutation induction on radiation dose has been found. High heterogeneity of cytogenetic parameters (aneuploidy and chromosome aberration frequency) has been shown in the mutants.

Green MH, Lowe JE, Delaney CA, Green IC. **Comet assay to detect nitric oxide-dependent DNA damage in mammalian cells**. Methods Enzymol 1996;269:243-66.

Guillen I, Lleonart R, Garcia Del Barco D, Martinez R, Herrera F, Morales A, Herrera MT, Morales R, De La Fuente J. **Reporter genes for transgenic fish experiments**. Biotecnol Apl 1996;13(4):279-83.
CBAC COPYRIGHT: CHEM ABS The study of regulatory sequences in transient expts. employing reporter genes produce valuable data to design gene constructs for trans genesis. In this report, Escherichia coli chloramphenicol acetyltransferase (CAT), hepatitis B surface antigen (BHsAg), E. coli beta-galactosidase and the green fluorescent protein (GFP) from the jellyfish Aqueorea victoria were assayed as reporter genes for in vivo transient expression in tilapia, common carp and zebrafish. In tilapia and common carp, an endogenous beta-galactosidase-like activity was found in embryos, and after hatching, it was localized in the posterior-ventral part of the fry. This beta-galactosidase-like activity could interfere with the use of lacZ as a reporter gene in expts. with tilapia and common carp when the expression is assayed in the early stages of development. In zebrafish, however, lacZ was successfully employed. The HBsAg and CAT genes gave reproducible results in the species tested, thus constituting a choice when a second reporter gene is needed as an internal control. Finally, the GFP provided a simple and powerful mean of monitoring transient gene expression in live zebrafish embryos.

Hee SS. **Mutagenesis and acute toxicity studies on saliva-leached components of chewing tobacco and simulated urine using bioluminescent bacteria**. ACS Symp Ser 1997;654:77-82.
CBAC COPYRIGHT: CHEM ABS A review with 11 refs. The task of minimizing expensive chem. analyses is usually attempted through bioassay techniques or tracers to signal the presence of compds. of interest before complete chem. anal. The Salmonella typhimurium reverse mutation assay has been the chief bioassay used. However only point mutations are detd., and metals and orgs. genotoxic through other mechanisms are not detected. The use of bioluminescent bacteria in acute (Photobacterium

phosphoreum) and genotoxic (Vibrio fischeri dark mutant) assays has been investigated for compds. in chewing tobacco that are available to simulated saliva and whose metabolites may be present in urine (investigated in simulated urines). Gas chromatog./mass spectrometry revealed that nicotine was the major compd. leached into simulated saliva. Nicotine was not genotoxic in the reverse mutation test using a dark mutant, unlike its metabolite, cotinine. Recommendations on the conditions that need to be controlled are provided for these tests.

Holbrook NJ, Liu Y, Fornace AJ Jr. **Signaling events controlling the molecular response to genotoxic stress**. EXS 1996;77:273-88.
Recently, much progress has been made in defining the signal transduction pathways mediating the cellular response to genotoxic stress. Multiple pathways involving several distinct MAP kinases (ERK, JNK/SAPK, and p38/HOG1) as well as the tumor suppressor protein p53 contribute to the response; the various pathways being differentially activated by particular genotoxic agents. Although both DNA damage and extranuclear events are important in initiating the response, recent evidence suggests the response is controlled primarily through events occurring at the plasma membrane, overlapping significantly with those important in initiating mitogenic responses. Attenuation of the responses appears to be largely controlled through feedback mechanisms involving gene products produced during the activation process.

Hornberg C, Macieuleviciute L, Seemayer NH. **[Comparative studies on the genotoxic effects of particular airborne pollutants on tracheal epithelial cells of the respiratory tract in different mammal species]**. Atemweg Lungenkr 1996;22(6):342-4. (Ger)
BIOSIS COPYRIGHT: BIOL ABS. RRM Research article human hamster.

Hornberg C, Maciuleviciute L, Seemayer NH. **Sister chromatid exchanges in rodent tracheal epithelium exposed in vitro to environmental pollutants**. Toxicol Lett 1996;88(1-3):45-53.
In our highly industrialized world, air pollution has become a major topic. The human respiratory tract is constantly exposed to air pollutants by inhalation. Besides gaseous pollutants airborne particulates are of great importance, containing a complex mixture of several hundred substances. The tracheobronchial epithelium is the major target site of airborne particulates as well as the origin of the most common cancer in man, the bronchogenic carcinoma. In our study we collected samples of airborne particulates in winter 1991 in the highly industrialized Rhine-Ruhr area (Germany) with a high-volume sampler on glass fiber filters. Airborne particulates were extracted with di-chloromethane and quantitatively transferred to dimethyl sulfoxide (DMSO) for tissue culture experiments. As target cells for genotoxicity testing we used cultures of rodent tracheal epithelial cells from the Syrian golden hamster and from the rat. Induction of "sister chromatid exchanges" (SCE) was utilized as a sensitive cytogenetic endpoint for evaluation of the genotoxic activity of extracts of airborne particulates. In presence of global extracts (GEX) we observed a dose-dependent, highly significant increase of SCE in tracheal epithelial cells of the Syrian golden hamster and of the rat. It is remarkable that even quantities of chemical substances equivalent to airborne particulates from less than 1 m3 of air were genotoxic. Results of this study and earlier reports demonstrate that rodent tracheal epithelial cells offer a reliable and sensitive in vitro model for genotoxicity testing of airborne particulates. Therefore, tracheal epithelial cells in vitro appear a meaningful alternative to other human and rodent cell culture systems which have been used for genotoxicity testing of air pollutants.

Hornberg C, Seemayer NH. **Induction of sister chromatid exchanges in rodent tracheal epithelial cells as a sensitive bioassay for detection of genotoxic activity of airborne particulates**. Exp Toxicol Pathol 1995;47(4):241-3.

Horst JP, Fritz HJ. **Counteracting the mutagenic effect of hydrolytic deamination of DNA 5-methylcytosine residues at high temperature: DNA mismatch N-glycosylase Mig.Mth of the thermophilic archaeon Methanobacterium thermoautotrophicum THF**. EMBO J 1996;15(19):5459-69.
Spontaneous hydrolytic deamination of DNA 5-methylcytosine residues gives rise to T/G mismatches which are pre-mutagenic lesions requiring DNA repair. For fundamental reasons, the significance of this and other processes lowering genetic fidelity must be accentuated at elevated temperatures, making thermophilic organisms attractive objects for studying how cells cope with thermal noise threatening the integrity of their genetic information. Gene mig of Methanobacterium thermoautotrophicum THF, an anaerobic archaeon with an optimal growth temperature of 65 degrees C, was isolated and its product (Mig.Mth; EC3.2.2-) shown to be a T/G-selective DNA thymine N-glycosylase with the properties required for counteracting the mutagenic effect of hydrolytic 5-meC deamination. The enzyme acts on T/G and U/G oppositions with similar efficiency; G/G, A/G, T/C and U/C are minor substrates; no other opposition of common nucleobases is attacked and no removal of U from single-stranded DNA is observed. Substrate preferences are modulated by sequence context. Together with the results presented here, one example of an enzyme directed against the hydrolytic deamination damage of 5-meC is known from each of the three phylogenetic kingdoms; entry into the repair pathway is glycosylytic in the eukaryotic and the archaeal case, whereas the eubacterial repair starts with an endonucleolytic DNA incision.

Hsu T, Deng FY. **Studies on the susceptibility of various organs of zebrafish (Brachydanio rerio) to benzo(a)pyrene-induced DNA adduct formation**. Chemosphere 1996;33(10):1975-80.
BIOSIS COPYRIGHT: BIOL ABS. The susceptibility of various fish organs to polycylic aromatic hydrocarbons(PAHs)-induced DNA adduct formation was studied in zebrafish using benzo(a)pyrene as the representative carcinogenic PAH. Following exposure of fish to waterborne BaP at 0.2 mg/L for 3 days and at 1 mg/L for 4 days, 32P-postlabelling analysis indicated that the adduct levels in intestine, liver, brain, and testis DNA were 13.3 : 1.2, 4.3 : 2.5, 3.8 : 0.5, and 0.2 : 0.1 adducts per 108 nucleotides, respectively. When zebrafish were treated with BaP at 0.02 mg/L for 3 days and at 0.1 mg/L for 4 days, a significant increase in the level of bulky adducts was detected only in intestine DNA (0.28 : 0.06 adduct/108 nucleotides), and no adduct spots were observed for DNA isolated from other organs. Our data suggest that the fish intestine is a more sensitive target organ than the liver for biomonitoring the presence of carcinogenic PAHs in the aquatic environment, especially when PAHs are present at low levels.

Hu Q, Hill RP. **Radiosensitivity, apoptosis and repair of DNA double-strand breaks in radiation-sensitive Chinese hamster ovary cell mutants treated at different dose rates**. Radiat Res 1996; 146 (6):636-45.

Keen J, Busby S. **Studies on the Escherichia coli melAB promoter**. Biochem Soc Trans 1996; 24 (2):246s.

Kevekordes S, Grahl K, Zaulig A, Dunkelberg H. **Nitro musk compounds: genotoxic activity: Genotoxicity testing of nitro musks with SOS-chromotest and the sister-chromatid exchange test**. Environ Sci Poll Res Int 1996;3(4):189-92.
BIOSIS COPYRIGHT: BIOL ABS. Five nitro musk compounds are widely used as fragrance ingredients in perfumes, lotions and detergents; as food additives in cigarettes and fish baits, and in such technical products as herbicide formulations and explosives. Several studies identified nitro musk compounds in aquatic environment samples, human milk and fat samples as highly lipophilic and persistent bioaccumulating environmental pollutants. To examine the compounds for genotoxic activity, musk xylene (1-tert.-butyl-3,5-dimethyl-2,4,6-trinitrobenzene), musk ketone (4-tert.-butyl-3,5-dinitro-2,6-dimethylacetophenone), musk ambrette (1-tert.-butyl-4-methyl-6-methoxy-3,5-dinitrobenzene), musk moskene (1,1,3,3,5-pentamethyl-4,6-di-nitroindane) and musk tibetene (1-tert.-butyl-3,4,5-trimethyl-2,6-dinitrobenzene) were tested for SOS inducing potency in the SOS chromotest with E. coli PQ37 and for sister-chromatid exchange inducing activities in human lymphocytes in vitro both in the presence and absence of an exogenous metabolizing system from rat liver S9-Mix. Nitro musks revealed no genotoxicity either in the SOS chromotest with E. coli PQ37 or in the sister-chromatid exchange test with human lymphocytes.

King RW, Glotzer M, Kirschner MW. **Mutagenic analysis of the destruction signal of mitotic cyclins and structural characterization of ubiquitinated intermediates**. Mol Biol Cell 1996;7(9):1343-57.
Mitotic cyclins are abruptly degraded at the end of mitosis by a cell-cycle-regulated ubiquitin-dependent proteolytic system. To understand how cyclin is recognized for ubiquitin conjugation, we have performed a mutagenic analysis of the destruction signal of mitotic cyclins. We demonstrate that an N-terminal cyclin B segment as short as 27 residues, containing the 9-amino-acid destruction box, is sufficient to destabilize a heterologous protein in mitotic Xenopus extracts. Each of the three highly conserved residues of the cyclin B destruction box is essential for ubiquitination and subsequent degradation. Although an intact destruction box is essential for the degradation of both A- and B-type cyclins, we find that the Xenopus cyclin A1 destruction box cannot functionally substitute for its B-type counterpart, because it does not contain the highly conserved asparagine necessary for cyclin B proteolysis. Physical analysis of ubiquitinated cyclin B intermediates demonstrates that multiple lysine residues function as ubiquitin acceptor sites, and mutagenic studies indicate that no single lysine residue is essential for cyclin B degradation. This study defines the key residues of the destruction box that target cyclin for ubiquitination and suggests there are important differences in the way in which A- and B-type cyclins are recognized by the cyclin ubiquitination machinery.

Kitaeva LV, Mikheeva EA, Shelomova LF, Shvartsman P Ya. **[Genotoxic effect of formaldehyde in somatic human cells in vivo]**. Genetika 1996;32(9):1287-90. (Rus)
BIOSIS COPYRIGHT: BIOL ABS. The genotoxic effect of formaldehyde (F) (chromosome aberrations in peripheral blood lymphocytes, micronucleated cells in buccal mucosa) was studied in workers manufacturing nitrogen fertilizer and exposed to F at, concentrations exceeding maximum permissible

ones for a working area (group 1); in workers at the Department of Normal Anatomy who handle moist anatomical preparations (group 2); and in students who attended anatomy lessons once (group 3). A pronounced F cytotoxic effect was found in groups 1 and 2. In lymphocytes obtained from individuals of group 1, in which frequency of chromosome aberrations exceeded the control level fourfold, metaphase plates were revealed only after 72 h of cultivation. A similar reduction of the statmokinetic index and an increase in chromosomal aberrations were observed after in vitro F treatment of lymphocytes. In groups 2 and 3, a four- to five-fold excess of micronucleated cells was found in buccal mucosa. In students, the number of micronucleated cells remained higher both 24 and 48 h after they handled moist formaline preparations in anatomy class for 40 min.

Kligerman AD, Doerr CL, Milholland VS, Tennant AH. **Cytogenetic effects of butadiene metabolites in rat and mouse splenocytes following in vitro exposures**. Toxicology 1996;113(1-3):336-40.
As a first step in investigating the genotoxic effects of the principal metabolites of 1,3-butadiene (BD) in both rats and mice, splenocytes (which have little mixed function oxidase activity) from each specimen were exposed to a series of concentrations of either 3,4-epoxy-1-butene (EB) (20 to 931 microM) or 1,2:3,4-diepoxybutane (DEB) (2.5 to 160 microM) for 1 h. The splenocytes were then washed, cultured, and stimulated to divide with concanavalin A, and metaphases were analyzed for the induction of sister chromatid exchanges (SCEs) and chromosome aberrations (CAs). In addition, cells from some experiments were taken after exposure but before culture, and subjected to the taken after exposure but before culture, and subjected to the single cell gel (SCG) assay to measure DNA damage in the form of DNA strand breakage and/or alkaline-labile sites. Initial studies indicate that EB does not induce cytogenetic damage in either rat or mouse G0 splenocytes. However, DEB was an extremely potent SCE- and CA-inducer in both species with no species differences apparent. Neither DEB nor EB produced any statistically significant DNA-damaging effects as measured by the SCG assay.

Kluwe WM. **The complementary roles of in vitro and in vivo tests in genetic toxicology assessment**. Regul Toxicol Pharmacol 1995;22(3):268-72.
Risk of genetic alteration (genetic toxicity) in humans as a consequence of exposure to exogenous agents is determined in large degree by the results of specific laboratory tests. Although the individual test procedures are uniform and standardized, there is often confusion when effects observed in vitro are not confirmed in vivo. This in vitro/in vivo difference is commonly misrepresented as demonstrating the insensitivity of in vivo genetic toxicology tests. Consideration of the mechanistic bases of the tests leads to a more rational interpretation: In vitro procedures, by avoiding pharmacokinetic limitations and many confounding interactions, are best able to detect the potential for an agent to affect genetic fidelity, while in vivo procedures, specifically because they are influenced by pharmacokinetics and competing reactions, are more suitable for determining the probability of genetic alterations occurring in an intact, dynamic organism. Expectations that in vivo test results should always confirm in vitro findings are unwarranted, as are comparisons of perceived sensitivities for detecting genetic toxicity. Human risk estimation should be based principally on the results of in vivo genetic toxicology tests.

Kreja L, Selig C, Plappert U, Nothdurft W. **Radiation-induced DNA damage in canine hemopoietic cells and stromal cells as measured by the Comet assay**. Environ Mol Mutagen 1996;27(1):39-45.
Using the single cell gel electrophoresis assay, the initial radiation damage in DNA of canine hemopoietic and stromal cells was measured following in-vitro radiation exposure. Buffy coat (BC) cells

prepared from canine bone marrow aspirates were used as a source of hemopoietic cells. Mixed stromal cells (SC) were harvested from an established adherent cell layer as well as from pure populations of bone marrow fibroblasts derived from fibroblastoid colony forming unit (CFU-F) colonies. Cells were irradiated with X-ray doses of 1 to 8 gray (Gy). Radiation exposure caused a significant increase in DNA damage with increasing doses of irradiation for bone marrow (BM) BC cells, but a relatively small increase for both populations of cultured stromal cells, SC and CFU-F. Even so, the tail movement (TM) values at any radiation dose were significantly different from control levels. The median values of the TM for BM cells and for SC and CFU-F showed a clear difference in DNA damage between hemopoietic cells and stromal cells. TM values for BM cells were clearly higher than those in both SC populations at any radiation dose studied. The radiation response of individual cells among the three different populations under study was heterogeneous. The TM values measured in normal cells showed nearly identical frequency distribution in all three cell populations. The greater DNA damage in a large fraction of BM cells was already noticed after the lowest dose of 1Gy and was very much pronounced after 8Gy. The authors suggest that the radiation induced damage in hemopoietic cells may be directly involved in the mechanism determining cell survival in an unknown way.

Kumari R. **Effectiveness and efficiency of physical, chemical and physico-chemical mutagens in M2 generation of Vicia faba L. var VH82-1**. J Nucl Agric Biol 1996;25(3):172-5.
BIOSIS COPYRIGHT: BIOL ABS. The mutagenic effectiveness, efficiency and efficacy Of gamma rays (10, 20 and 30 Krad), EMS (0.10 and 0.40%) their combination treatments 10, 20, 30 Krad-gamma rays + 0.10% EMS and 10, 20, 30 Krad gamma rays + water were studied on Vicia faba L. var. VH82-1. Effectiveness of mutagens measured by the rate of mutation as related to unit dose of mutagens, was found to be in order of EMS> gamma rays + EMS> gamma rays + water gamma rays. Efficiency of mutagens as estimated by the rate of mutation in relation to other biological effects induced usually a measure of damage in MI was gamma rays + water> gamma rays + EMS> gamma rays> EMS (MP/L, MP/S, MP/CA). Gamma rays 20 Krad sole or combined either with EMS or water were found to be most effective and efficient doses and 0.10% EMS was most effective concentration.

Lafreniere RG, Rochefort DL, Kibar Z, Fon EA, Han F, Cochius J, Kang X, Baird S, Korneluk RG, et al. **Isolation and characterization of GT335, a novel human gene conserved in Escherichia coli and mapping to 21q22.3**. Genomics 1996;38(3):264-72.
CBAC COPYRIGHT: CHEM ABS As part of efforts to identify candidate genes for disorders mapped to 21q22.3, a 405-kb cosmid contig was constructed encompassing 5 tightly linked markers mapping to this region. A subset of these cosmids was used to identify cDNA fragments by the method of hybrid selection. The cDNA sequence of one such gene (GT335) mapping to this region is presented. The gene is expressed as a 1.7-kb transcript predominantly ion heart and skeletal muscle, potentially displays alternate splicing, and is predicted to encode a protein 268 amino acids in length. GT335 spans an estd. 13 kb of genomic DNA and is split into 7 exons. Five of the 6 introns conform to the GT...AG consensus for intronic splice junctions; the sixth contains nonconventional (AT...AC) intronic junctions. This gene was screened for single-base pair mutations using single-strand conformation polymorphism and sequence anal. of both cDNA and genomic DNA from a no. of unrelated individuals and several sequence variations were identified, 2 of which cause conservative amino acid substitutions. This gene is well conserved evolutionarily, with homologs identified in zebrafish and Escherichia coli, suggesting

that it plays an important role in basic cellular metab.

Lazutka JR. **Genetic toxicity of cytokines**. Mutat Res 1996;361(2-3):95-105.
Review of the literature shows that such cytokines as human interferons alpha and gamma, tumor necrosis factor alpha, epidermal growth factor and interleukin-2 may exhibit genotoxic properties in human peripheral blood lymphocyte cultures. For all above cytokines, except interleukin-2, parabolic-like relationship between the dose and the frequency of sister chromatid exchanges was found. Although the mechanisms of these genotoxic actions remain largely unknown, generation of free radicals or interaction with enzymes such as DNA topoisomerase II may be suspected. Human interferon alpha also may be considered as an antimutagenic compound in human cells. Human tumor necrosis factor alpha has been reported to enhance cytotoxicity and DNA fragmentation produced by DNA topoisomerase II-targeted anticancer drugs. At the same time, it has some radio- and chemoprotective properties in vitro and in vivo. Despite these facts, the question about genotoxicity of cytokines is not answered. Some problems must be resolved before receiving the final answer. First, much more cytokines must be tested for their genotoxic activity. Second, appropriate test-systems must be designed. Third, genotoxicity studies of cytokines must account for cytokine interaction in the cytokine network as well as for such cytokine-induced effects as cytotoxicity and apoptosis. Fourth, in each case, it is necessary to have experimental evidence that observed genotoxic effects were caused by cytokine under investigation and not by the other factors.

Li CS, Lin RH. **Evaluation of low-dosage environmental mutagens with a long-term, cultured epithelial cell line**. Bull Environ Contam Toxicol 1996;56(6):919-25.
A long term cultured epithelial cell line was used to evaluate low dosage environmental mutagens. Human epithelial cells (HS1 cells) were exposed to benz(a)anthracene (56553) (BaA) or benzo(a)pyrene (50328) (BaP) in-vitro; the frequency of HGPRT-mutants was then determined via limiting dilution analysis in 6-thioguanine (6-TG) containing medium. Test concentrations of 1 and 10 nanograms/ milliliter were selected based on preliminary range finding cytotoxicity assays. The mutagenic assay proved to be very sensitive; mutagenic effects were detected in long term cultured epithelial cell lines even at very low concentrations of BaA and BaP. Other advantages of the described assay over Ames tests and other animal tissue cell assays included diminished transformation frequency, more direct application to human health, and particular emphasis on the respiratory epithelium as a target of inhaled environmental chemicals. The authors concludethat the described epithelial cell assay system may be a very useful tool for evaluating the mutagenic effects of environmental chemicals on the human respiratory tract; further studies are needed to determine whether this technique can also be used to predict carcinogenicity.

Liu S, Dixon K. **Induction of mutagenic DNA damage by chromium(VI) and glutathione**. Environ Mol Mutagen 1996;28(2):71-9.
BIOSIS COPYRIGHT: BIOL ABS. Certain chromium (Cr) compounds are known to be carcinogenic in humans and mutagenic in cell culture. However, the mechanism of Cr mutagenesis is not well understood. It appears that intracellular reduction of Cr by agents such as glutathione plays a role in the induction of DNA damage. We have used a simian virus 40-based shuttle vector to investigate the relationship between chromium-induced DNA damage and Cr mutagenicity. The treatment of the plasmid pZ189 with Cr(VI) plus glutathione (GSH) induced DNA strand breaks and reduced the plasmid

biological activity, whereas Cr(III) treatment with or without GSH did not give rise to such DNA damage. When Cr(VI)/GSH- or Cr(III)/GSH-treated pZ189 was replicated in mammalian cells, a dose-dependent increase in mutant frequency was observed with Cr(VI)/GSH-treated pZ189, but not with Cr (III)/GSH-treated plasmid. About 43% of the mutants from Cr(VI)/GSH-treated pZ189 were deletion mutants. The remainder were base substitution mutants, mostly GC - AT transitions and GC - TA transversions. This pattern of mutagenesis is similar to that observed with other agents that cause oxidative DNA damage such as ionizing radiation and H2O2. These results support the hypothesis that Cr mutagenesis can be induced by the generation of reactive oxygen intermediates during the reduction of Cr(VI) by glutathione.

Liu ZG, Baskaran R, Lea-Chou ET, Wood LD, Chen Y, Karin M, Wang JY. **Three distinct signalling responses by murine fibroblasts to genotoxic stress**. Nature 1996;384(6606):273-6.
Genotoxic stress triggers signalling pathways that mediate either the protection or killing of affected cells. Whereas induction of p53 involves events in the cell nucleus, the activation of transcription factors AP-1 and NF-kappaB by ultraviolet radiation is mediated through membrane-associated signalling proteins, ruling out a nuclear signal. An early event in AP-1 induction by ultraviolet radiation is activation of Jun kinases (JNKs), which mediate the induction of the immediate-early genes c-jun and c-fos. The JNKs have also been proposed to mediate the apoptopic response to genotoxins. The non-receptor tyrosine kinase c-Abl is also activated by genotoxic stress. To understand the relationship between these events, we compared the activation of p53, JNK and c-Abl by several DNA-damaging agents in murine fibroblasts. We found that whereas p53 was induced by every genotoxic stimulus tested, c-Abl was activated by most stimuli except ultraviolet irradiation and JNK was strongly stimulated only by ultraviolet light and the alkylating agent methyl methanesulphonate. Activation of JNK by this alkylating agent was normal in c-Abl-null cells but was reduced in c-Src-null cells. Unlike p53 induction, c-Abl activation occurs in the S phase of the cell cycle and does not affect cell proliferation. These findings show that signals generated by genotoxins are transduced by multiple, independent pathways. Only p53 appears to be a universal sensor of genotoxic stress.

Longo JA, Nevaldine B, Longo SL, Winfield JA, Hahn PJ. **An assay for quantifying DNA double-strand break repair that is suitable for small numbers of unlabeled cells**. Radiat Res 1997;147(1):35-40.
A system based on pulsed-field gel electrophoresis (PFGE) is described which measures the induction and repair of DNA double-strand breaks (DSBs) in a biologically relevant X-ray dose range (below 10 Gy) using as few as 125 cells per time. This system was used to measure repair in cells of a freshly obtained human glioblastoma multiforme tumor. No prelabeling of the cells is required, and many different cell types can be studied using this system. Under the pulsed-field conditions used, DNA in the range of 2 to 6 Mb enters the PFGE gel and forms an upper compression zone directly under each well. To quantify the DSBs after electrophoresis, the DNA was transferred to nylon membranes and hybridized with 32P-labeled chromosomal DNA. Phosphor screens were exposed to the membranes and scanned on a phosphor imager. The kinetics of induction and repair was determine by measuring the amount of DNA in the compression zones compared to the amount in the wells. EMT-6 cells were used to demonstrate this method. Induction of DSBs by doses of 0-7.5 Gy X rays was assayed using approximately 12,500 cells per dose and was shown to be linear. Double-strand breaks from 1 Gy were

detected above background. To determine a lower limit of the number of cells that could be used to measure DSB repair, cells were embedded in agarose at decreasing concentrations per plug, exposed to 7.5 Gy X irradiation and allowed to repair at 37 degrees C for up to 60 min. DNA from approximately 12,500, 1,250 and 125 cells per time was loaded and subjected to PFGE. The average fast-repair half-time was 3 min and the slow-repair half-time was 35 min. The kinetics of DSB repair in glioblastoma multiforme cells was also determined using this system. Agarose plugs were prepared from a cell suspension, irradiated with 7.5 Gy X rays and allowed to repair for up to 90 min. DNA from approximately 1,250 tumor cells was electrophoresed and analyzed as described above for EMT-6 cells. For this particular tumor, approximately 75% of the induced DSBs were repaired after 90 min. Data presented show that this PFGE-based system is an extremely sensitive method for measuring DSB induction and repair after low doses of X rays using very few cells.

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.
Among the main DNA-reactive metabolites of 1,3-butadiene (BD), both 1,2:3,4-butadiene diepoxide (BDE) and 1,2-epoxy-3-butene (BME) have been reported in mice and rats exposed to BD, but blood and tissue levels of these metabolites are much higher in mice than in rats under similar exposure conditions. BDE, being more reactive and genotoxic than BME, is thought to be responsible for the greater susceptibility of mice to BD carcinogenicity. While BDE is a DNA-alkylating agent and some BDE adducts have been characterized, no sufficiently sensitive method has been reported for studying BDE-DNA binding in vivo. In the present investigation, a modified dinucleotide/monophosphate version of the 32P-postlabeling assay was applied to detect BDE-DNA adducts, which were prepared by reacting BDE with calf thymus DNA or deoxyribooligonucleotides [(AC)10, (AG)10, (CCT)7 and (GGT)7] in vitro or with skin DNA of mice in vivo upon topical treatment. Optimal resolution by 2-D PEI-cellulose TLC of the highly polar 5'-monophosphate adducts was achieved at +4 degrees C using 0.3 M LiCI (DI) and 0.4 M NaCl, 0.04 M H3BO3, pH 7.6 (D2). The profiles of the 32P-postlabeled adducts were similar for calf thymus and skin DNA, with 3 major spots being detected. Adducts obtained in in vitro and in vivo experiments were compared by re- and cochromatography in 4 or 5 different solvents, and these experiments provided evidence that corresponding BDE adducts, for the most part, were identical and represented adenine derivatives. Guanine adducts were not detected by this method although literature data indicate their formation. Quantitatively, the assay responded linearly to adduct concentration, as shown in an experiment where BDE-modified skin DNA was serially diluted up to 81-fold with control DNA. The limit of detection was approximately 1 adduct in 10(8) normal nucleotides. Further, in an in vivo dosimetry study, skin DNA from groups of 8 individual mice treated with different doses of BDE (1.9, 5.7, 17, 51 and 153 mumol/mouse) for 3 days exhibited a linear relationship (r > or = 0.992) between adduct levels and dose. The results suggest that the 32P-postlabeling assay described herein will have utility in mechanistic studies and biomonitoring of DNA adduct formation from BDE and possibly other polar epoxides.

Majtan V, Majtanova L. **Effect of some organic ammonium salts and amine oxides in the SOS chromotest**. Pharmazie 1996;51(10):753-5.
The effect of two organic ammonium salts, (1-methyldodecyl)trimethylammonium bromide (ATDBr)

and tetramethylammonium bromide (TMABr), as well as that of two amine oxides, (1-methyl-dodecyl) dimethylamine oxide (ATDNO) and trimethylamine oxide (TMANO) on the induction of the SOS system in Escherichia coli PQ 37 (SOS chromotest) was examined. The compound with the long alkyl chain in the molecule (ATDBr) showed genotoxic activity in all concentrations tested. In the second group of the amphiphilic compounds tested, amine oxides, no effect of the length of the alkyl chain upon the studied activity was ascertained.

Malaveille C, Hautefeuille A, Pignatelli B, Talaska G, Vineis P, Bartsch H. **Dietary phenolics as anti-mutagens and inhibitors of tobacco-related DNA adduction in the urothelium of smokers**. Carcinogenesis 1996;17(10):2193-200.
Human urine is known to contain substances that strongly inhibit bacterial mutagenicity of aromatic and heterocyclic amines in vitro. The biological relevance of these anti-mutagens was examined by comparing levels of tobacco-related DNA adducts in exfoliated urothelial cells from smokers with the anti-mutagenic activity in corresponding 24-h urine samples. An inverse relationship was found between the inhibition of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-mutagenicity by urine extracts in vitro and two DNA adduct measurements: the level of the putatively identified N-(deoxyguanosine-8-yl)-4-aminobiphenyl adduct and the total level of all tobacco-smoke-related carcinogen adducts including those probably derived from PhIP. Urinary anti-mutagenicity in vitro appears thus to be a good indicator of the anti-genotoxicity exerted by substances excreted in urine, that protect the bladder mucosal cells (and possibly other cells) against DNA damage. These substances appear to be dietary phenolics and/or their metabolites because (i) the anti-mutagenic activity of urine extracts (n = 18) was linearly related to their content in phenolics; (ii) the concentration ranges of these substances in urine extracts were similar to those of various plant phenols (quercetin, isorhamnetin and naringenin) for which an inhibitory effect on the liver S9-mediated mutagenicity of PhIP was obtained; (iii) treatment of urines with beta-glucuronidase and arylsulfatase enhanced both anti-mutagenicity and the levels of phenolics in urinary extracts; (iv) urinary extracts inhibited noncompetitively the liver S9-mediated mutagenicity of PhIP as did quercetin, used as a model phenolics. Several structural features of the flavonoids were identified as necessary for the inhibition of PhIP and 2-amino-3,8-dimethylimidazo [4,5-f]quinoxiline mutagenicity. Fractionation by reverse-phase HPLC and subsequent analysis of two urinary extracts, showed the presence of several anti-mutagenic substances and phenolics; more lipophilic phenolics displayed the highest specific inhibitory activity. This suggests that enzymatic conversion of dietary flavonoids into their more lipophilic and anti-mutagenic O-methylcatechol derivatives, as noted for quercetin, may occur in vivo in man. Onion, lettuce, apples and red wine are important sources of dietary flavonoids which are probably responsible for the anti-mutagenicity associated with foods and beverages. After HPLC fractionation of urinary extracts, the distribution profile of anti-mutagenic activity corresponded roughly to that of onion and wine extract combined. Our study strongly suggests that smokers ingesting dietary phenolics, probably flavonoids, are partially protected against the harmful effects by tobacco carcinogens within their bladder mucosal cells. This protective effect of dietary phenolics against the cancer of the bladder (and possibly other sites) should be verified and explored as a part of a chemoprevention strategy.

Manjanatha MG, Lyn-Cook LE, Culp SJ, Beland FA, Heflich RH, Aidoo A. **Lymphocyte mutant frequency in relation to DNA adduct formation in rats treated with tumorigenic doses of the**

mammary gland carcinogen 7,12-dimethylbenz(a)anthracene. Mutat Res 1996;357(1-2):89-96.
BIOSIS COPYRIGHT: BIOL ABS. The ability of the rat lymphocyte hprt assay to detect tissue-specific carcinogens was evaluated using 7,12-dimethyl-benz(a)anthracene (DMBA) administered under conditions that result in mammary gland tumors. Fifty-day-old female Sprague-Dawley rats were given single doses of 5 and 20 mg/kg DMBA by gavage, and the frequency of 6-thioguanine-resistant (TGr) T-lymphocytes was measured over a period of 21 weeks. A time- and dose-dependent increase in mutant frequency was found, with a maximum frequency found 9-15 weeks after treatment with 20 mg/kg of DMBA. Rats were also dosed with 1, 2.5, 5, 10, 15 and 20 mg/kg of DMBA and assayed for TGr mutant frequency 10 weeks after treatment. A significant linear dose-response was found, with all the DMBA doses resulting in significant increases in mutant frequency. To determine whether or not DMBA-induced mutants in rat lymphocytes reflected the DNA damage in the target tissue, rats were treated with 5 and 20 mg/kg of DMBA and spleen lymphocytes and mammary gland tissue were assayed for DNA adduct formation 1, 3 and 7 days later. A similar pattern of 32P-postlabeled adducts, involving both dG and dA nucleotides, was found in DNA from both the target tissue and the surrogate lymphocytes. Adduct formation was dose responsive.

Mano H, Roa I, Araya JC, Ohta T, Yoshida K, Araki K, Kinebuchi H, Ishizu T, Nakadaira H, Endoh K, et al. **Comparison of mutagenic activity of bile between Chilean and Japanese female patients having cholelithiasis**. Mutat Res 1996;371(1-2):73-7.
The mutagenic activity of bile was compared between Chilean and Japanese female patients having cholelithiasis by the Ames assay using Salmonella typhimurium tester strain TA98 in the presence of S9 mix with blue rayon adsorption technique. A reason for conducting the present investigation is that Chile and Japan have the highest mortality rates for the gallbladder cancer (GBC) in the world. Of 24 bile samples collected in Chile, 20 (83.3%) samples showed mutagenicity. In the case of Japanese bile, 21 (80.8%) of 26 and 5 (19.2%) of 26 cases were mutagenic in samples from high- and low-risk areas for GBC, respectively. Therefore, both the Chilean and the Japanese samples collected in high-risk areas showed higher mutagenic rates than the Japanese ones in a low-risk area, with a statistical significance ($p < 0.001$), chi-square test). The average number of revertant colonies were 128 +/- 92 (mean +/- SD), 62 +/- 14 and 66 +/- 13, respectively, when the blue rayon extracts of 200 microliters bile were applied to the Ames test. Thus, Chilean bile had a tendency to show a higher mutagenic activity than Japanese.

Mathews CZ, Sjoeberg B, Karlsson M. **Cloning and sequencing of cDNAs encoding ribonucleotide reductase from zebrafish Danio rerio**. Mol Mar Biol Biotechnol 1996;5(4):284-7.
CBAC COPYRIGHT: CHEM ABS The authors have cloned and sequenced cDNAs coding for the R1 and R2 proteins of ribonucleotide reductase from zebrafish (Danio rerio). This ribonucleotide reductase shows high amino acid sequence identity to those of other vertebrates. The R1 cDNA has a coding sequence of 2382 bp, yielding a 794 amino acid protein, and the R2 cDNA has a coding sequence of 1158 bp, yielding a 386 amino acid protein. The zebrafish R1 shows 94% similarity and R2 shows 91% similarity to the human R1 and R2, resp. The similarity extends to intron positions, of which the equiv. of mouse R2 intron 3 has been studied.

Matsui M, Matsui K, Kawasaki Y, Oda Y, Noguchi T, Kitagawa Y, Sawada M, Hayashi M, Nohmi T, Yoshihira K, et al. **Evaluation of the genotoxicity of stevioside and steviol using six in vitro and one in vivo mutagenicity assays**. Mutagenesis 1996;11(6):573-9.

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

McGregor DB, Riach C, Cattanach P, Edwards I, Shepherd W, Caspary WJ. **Mutagenic responses of L5178Y mouse cells at the tk and hprt loci**. Toxicol In Vitro 1996;10(5):643-7.
BIOSIS COPYRIGHT: BIOL ABS. The expression of multiple recessive genes by aberrant mitotic lesions plays a major part in carcinogenesis. These lesions include intragenic mutations as well as chromosomal lesions. An in vitro model for studying carcinogenesis should respond to all these lesions. Mutagenesis studies that target hemizygous loci may not be useful in studying chromosomal mechanisms because lesions incorporating essential genes already missing on the inactive, homologous chromosome may be lethal to the cell. Cells heterozygous at the selectable gene may survive. Using L5178Y mouse cells, we compared the mutagenic responses at the heterozygous tk and hemizygous hprt loci to four chemicals-benzidine dihydrochloride, diglycidylresorcinol ether, nitrofen and p-benzoquinone dioxime. None of the compounds induced clear positive responses at the hprt locus. In contrast, all the compounds induced clear or marginal mutagenic responses at the tk locus. These data are consistent with the expectation that heterozygous loci can detect lesions that are refractory to hemizygous loci.

Mersch-Sundermann V, Emig M, Reinhardt A, Helbich HM. **[Co-genotoxic potential of PCB mixtures from pediatric fatty tissue]**. Gesundheitswesen 1996;58(7):400-5. (Ger)
In the present study a mixture containing the 11 PCB major components identified in fatty tissues of children was examined for its potency to enhance the toxification of pregenotoxicants (cogenotoxicity) in the liver. As a basis for the study GC/MS PCB analyses of 207 fatty tissue samples of children were used. The PCB mixture was produced on this basis. As a model for the identification of the cogenotoxic potency of the PCB mixtures an in vivo/in vitro enzyme induction assay was developed. The goal of the study was to clarify the question, whether the in vivo pretreatment of rats with a complex PCB pattern derived from children led to a synergism of cogenotoxicants and pregenotoxicants with regard to the enhancement of the in vitro toxification of benzo[a]pyrene (B[a]P) and 2-aminoanthracene (2-AA) to DNA reactive metabolites. Using the SOS-Chromotest as the in vitro part of the induction assay, all liver

enzyme fractions of PCB pretreated rats (S9PCB) showed an increase of the toxification of the pregenotoxicants B[a]P and 2-AA in comparison to enzyme factions of untreated animals (S9(0)). With regard to the reactivity pattern it may be supposed that the PCB mixture probably induced cytochrome P450-dependent oxigenases of the subclasses CYP1A1 and CYP1A2. Additionally, it seems to be of interest that the use of S9(0) fractions did not lead to any or only to weak toxification of B[a]P and 2-AA. Thus, a synergism of cogenotoxicants and pregenotoxicants could be confirmed. PCB could be identified in fatty tissues of children in amounts up to 1 mg/kg. Additionally, pregenotoxicants like polycyclic aromates, mycotoxins and/or aminocontaining compounds, are available in almost all environmental sources. Therefore, from the present point of view, a genetic risk caused by PCB in humans (children) cannot be excluded.

Miklos K, Hassanien MA, Geza T, Erzsebet A, Alan P. **Single-cell gel electrophoresis (Comet assay) for rapid detection of genotoxic materials**. Egeszsegtudomany 1996;40(3):274-82. (Hun)
CBAC COPYRIGHT: CHEM ABS The effects of CdCl2 on rat bronchoalveolar macrophages was measured by single cell gel electrophoresis (Comet assay), esp. suitable to detect single strand DNA breaks. The cells were treated with 0.03-0.30 muM/mL CdCl2. The results obtained after staining the cells with ethidium bromide were evaluated by (i) using the Colormorph image analyzer program or (ii) by counting the fraction of intact cells i.e. cells with undamaged DNA in their nuclei. The significant increase in the tail moments, calcd. by the Coloumorph, indicated single strand DNA breaks caused by CdCl2 but similar results were obtained by counting the intact cell nuclei (a 76% decrease was obsd. at 0.3 muM/mL CdCl2, the last subtoxic concn. of the chem.). Only a small (24%) fraction of the cell suspension was really sensitive to the genotoxic effect of the CdCl2, although the identity of this cohort of cells has not been exactly defined.

Miller DL, Thomas RM. **The role of cavitation in the induction of cellular DNA damage by ultrasound and lithotripter shock waves in vitro**. Ultrasound Med Biol 1996;22(5):681-7.
The induction of DNA strand breaks in Chinese hamster ovary cells was measured with the comet assay after continuous wave ultrasound or lithotripter shock wave exposure. Cell lysis and hydrogen peroxide production were measured to gauge the level of inertial cavitation activity. Significant DNA damage was found after 2.17-MHz ultrasound exposure at 37 degrees C to 0.82 MPa for 2 min or 4 min, and to 0.58 MPs for 4 min. A significant portion of the damage induced at the 0.82-MPa level was repaired by the cells when warmed. Neither exposure to 500 or 1000 shock waves at 37 degrees C in a thin-walled tube, nor exposure to 1000, 1500 or 2000 shock waves at 25 degrees C in a polyethylene pipette bulb produced a significant effect, when the flash of light from the spark-gap discharge was blocked. This finding was consistent with the generally lower lysis and hydrogen peroxide production by the shock wave exposure.

Mirkova E. **[The genotoxicity and carcinogenic potential of gastrofenzin]**. Eksp Med Morfol 1994;32 (3-4):57-68. (Bul)
Genotoxicity of the Bulgarian drug gastrophensin was studied by using a battery of two genotoxicity assays in vitro - Salmonella/mutation assay and in vivo - the rodent bone marrow micronucleus test. Mutagenicity of water solution of gastrophensin towards Salmonella in vitro - the rodent bone marrow micronucleus test. Mutagenicity of water solution of gastrophensin towards Salmonella in vitro was tested in five mutant, histidine auxotrophic strains - TA 1535, TA 1537, TA 1538, TA 98 and TA 100

without and in the presence of metabolic activation (+/- S9) at concentration of 0.4, 2 and 10 mg center dot ml-1. Gastrophensin did not induce mutagenic response in the Salmonella/mutation assay in a range of tested concentrations in both series of assays (+/- S9). Gastrophensin did not induce micronuclei in bone marrow cells of male C57Bl6 mice at 24, 48 and 72 hours after single oral treatment with 236 mg center dot kg-1 (80% DL50 oral, mice) and 118 mg center dot kg-1 (40% DL50 oral, mice). Based on the present data a conclusion of the lack of mutagenicity and of carcinogenic potency of gastrophensin was made.

Mizuno M, Kato T, Koyama K. **An analysis of mutagens in the contents of the biliary tract in pancreaticobiliary maljunction**. Surg Today 1996;26(8):597-602.
A reflux of pancreatic juice into the biliary tract due to pancreaticobiliary malfunction has been considered to be an important factor in the development of biliary tract carcinogenesis. It is known that the contents of the biliary tract contain not only activated pancreatic enzymes but also certain mutagens. The purpose of this study was thus to isolate and identify such mutagenic substances. A 1:1 mixture of the control bile and pancreatic juice was mixed with bovine enterokinase (10 mg/ml), and the mixture was incubated at 37 degrees C for 12 h. The mixture was demonstrated to be reproducibly mutagenic by the Ames test. The mutagenic substances in these mixtures, which were separated using organic analysis, were found to be included in the water-soluble fraction and to contain amino acids. Mutagenic substances are thought to have a molecular weight of 1,500-3,500 and to be a complex of low-molecular-weight, stable, mutagenic substances including amino acids and peptides.

Moore LE, Warner ML, Smith AH, Kalman D, Smith MT. **Use of the fluorescent micronucleus assay to detect the genotoxic effects of radiation and arsenic exposure in exfoliated human epithelial cells**. Environ Mol Mutagen 1996,27(3):176-84.
The genotoxic effects of arsenic (7440382) and ionizing radiation in exfoliated human epithelial cells were examined. Buccal cells were collected from a 29 year old male, 5 days a week, for 9 weeks (wk), before, during and 3wk after he was exposed to 6,500 rads of photon radiation treatment for a salivary gland tumor. Exfoliated bladder cells were also collected from 18 persons chronically exposed to drinking water contaminated with arsenic. The contaminated water contained arsenic concentrations above 500 micrograms per liter (microg/l). Water consumed by control subjects contained less than 10microg/l arsenic. After collection, the buccal and bladder cells were scored for centromere positive (MN+) and centromere negative (MN-) micronuclei using the fluorescent micronuclei (FMN) assay modified with a biotin labeled alpha satellite probe, specific for human centromeres. Irradiation of the cancer patient caused a 16.6 fold increase in buccal cells with micronuclei compared to the preradiotherapy value. Of the buccal cells, 89.5 to 92.3% were MN-, indicating that the micronuclei resulted from chromosome breaks. Three weeks after therapy ended, the percentage of micronucleated buccal cells returned to the control value. The mean proportion of micronucleated bladder cells seen in the subjects drinking arsenic contaminated water was significantly higher than in the controls, 0.28 versus 0.16%. The proportion of MNand MN+ cells in the exposed subjects was 1.65, and 1.37 times that in the controls, respectively. This finding suggested that arsenic may be both clastogenic and weakly aneuploidogenic in-vivo. Induction of MN+ and MNbladder cells was significantly greater in male subjects than in females. Induction of MN+ and MNbladder cells was significantly, positively associated with urinary excretion of total and inorganic arsenic. The authors conclude that the FMN assay with

centromeric probes can be used to elucidate the mechanism of micronuclei formation in epithelial tissues.

Mozhaeva TE. **[Problems in the study of mutagenic effects of environmental factors (review)]**. Gig Sanit 1996;(5):38-40. (Rus)

Mueller SO, Eckert I, Lutz WK, Stopper H. **Genotoxicity of the laxative drug components emodin, aloe-emodin and danthron in mammalian cells: Topoisomerase II mediated?** Mutat Res 1996;371 (3,4):165-73.
CBAC COPYRIGHT: CHEM ABS 1,8-Dihydroxyanthraquinones are under debate as plant-derived carcinogens that are found in laxatives, food colors, and possibly vegetables. Published genotoxicity data are controversial, and so three of them (emodin, danthron and aloe-emodin) were tested in a no. of in vitro assay systems. All three compds. induced tk-mutations in mouse lymphoma L5178Y cells. Induction of micronuclei also occurred in the same cell line, and was dose-dependent, with the potency ranking being danthron > aloe-emodin > emodin. In a DNA decatenation assay with a network of mitochondrial DNA of C. fasciulata, all three test compds. inhibited the topoisomerase II-mediated decatenation. Danthron and aloe-emodin, but not emodin, increased the fraction of DNA moving into comet tails when tested at concns. around 50 muM in single-cell gel-electrophoresis assays (SCGE; comet assay). Comet assays were also used in modified form to det. whether pretreatment of the cells with the test compds. would reduce the effects of etoposide, a potent topoisomerase II inhibitor. All three test chems. were effective in this pretreatment protocol, with danthron again being the most potent. Given clearcut evidence of their genotoxic activity, further research on the human cancer risk of these compds. may be warranted.

Mure K, Takeshita T, Takeuchi T, Morimoto K. **Urinary mutagens and lifestyle factors**. Prev Med 1996;25(5):569-74.
BACKGROUND: Lifestyle determines the amount of exposure to environmental carcinogens/mutagens. We examined the relationship between various lifestyle factors and the urinary level of mutagens, which reflects both exposure dose and metabolism of these carcinogens/ mutagens. METHODS: Twenty-four-hour urine specimens obtained from 69 males were subjected to blue rayon extraction, after which the elutions were fractionated by carboxymethyl cellulose column chromatography. The mutagens were measured by using an S9-mediated Salmonella mutagenicity test. The subjects were classified into three groups according to their total scores on a questionnaire regarding eight health practices: cigarette smoking, alcohol drinking, nutritional balance, eating breakfast, sleeping hours, working hours, physical exercise, and mental stress. RESULTS: Compared with those with poor health practice, subjects with good health practice showed a significantly lower urinary level of mutagens in all chromatography fractions, as well as in the acid- and alkali-elutable fractions. Cigarette smoking and nutritional balance were the most highly correlated factors. CONCLUSIONS: Our findings show that poor health practices increase the urinary level of mutagens, suggesting that a healthy lifestyle reduces exposure to carcinogens/ mutagens and may reduce cancer risk.

Nelson E. **Laboratory probing of oncogenes from human liquid and solid specimens as markers of exposure to toxicants**. Crit Rev Toxicol 1996;26(5):483-549.

Recent discoveries regarding the mechanistic role of oncogenes and tumor suppressor genes in cancer development have opened a new era of molecular diagnosis. It has been observed repeatedly that genetic lesions serve as tumor markers in a broad variety of human cancers. The ras gene family, consisting of three related genes, H-ras, K-ras, and N-ras, acquires transforming activity through amplification or mutation in many tissues. If not all, then most types of human malignancies have been found to contain an altered ras gene. Because the ras oncogenes actively participate in both early and intermediate stages of cancer, several highly specific and sensitive approaches have been introduced to detect these genetic alterations as biomarkers of exposure to carcinogens. There is also mounting evidence that implicate chemical-specific alterations of the p53 tumor suppressor gene detected in most human tumors. Therefore, it seems a reliable laboratory approach to identify both altered p53 and ras genes as biomarkers of human chronic or intermittent exposure to toxicants in a variety of occupational settings.

Nestmann ER, Bryant DW, Carr CJ. **Toxicological significance of DNA adducts: summary of discussions with an expert panel**. Regul Toxicol Pharmacol 1996;24(1 Pt 1):9-18.

Obata M, Nishimori H, Ogawa K, Lee GH. **Identification of the Par2 (Pulmonary adenoma resistance) locus on mouse chromosome 18, a major genetic determinant for lung carcinogen resistance in BALB/cByJ mice**. Oncogene 1996;13(8):1599-604.
The A/J mouse strain is 14 times more susceptible to urethane-induction of lung carcinogenesis than the BALB/cByJ strain (BALB). The relative resistance of BALB is dominant over the high sensitivity of A/J, since (BALBxA/J)F1 mice are phenotypically similar to the parental BALB mice. BALB mice must thus possess modifier genes suppressing phenotypic expression of the Pas (Pulmonary adenoma susceptibility) genes, which are known to be dominant genetic determinants for lung carcinogenesis in A/J mice. In order to genetically dissect the dominant resistance of the BALB mouse, we performed a linkage analysis to chromosomally map modifier genes by using 130 (A/JxBALB)F1xA/J backcross mice. Each backcross mouse was injected i.p. with urethane (1 mg/g bw) at 6 weeks of age and lung tumors were enumerated after 120 days. When the backcross mice were genotyped at multiple simple sequence repeat marker loci distributed on all the chromosomes, a significant linkage between the presence of a BALB allele and resistance to lung tumor induction was found on distal chromosome 18 (maximum LOD = 12.2). Thus, distal chromosome 18 of the BALB mouse contains a modifier gene for lung carcinogenesis: The locus, designated Par2 (Pulmonary adenoma resistance), accounted for 38% of the phenotypic variance in the backcross population, indicating a major role in protection against lung tumor development.

Pagano P, De Zaiacomo T, Scarcella E, Bruni S, Calamosca M. **Mutagenic activity of total and particle-sized fractions of urban particulate matter**. Environ Sci Technol 1996;30(12):3512-6.
BIOSIS COPYRIGHT: BIOL ABS. Total or inhalable (PM10) particulate matter is monitored as a quality air criteria. Airborne particles deposit onto the differently sensitive biological tissues of the respiratory tract depending on their size. So it is very important to know the mutagenic activity (index of potential carcinogenicity) of the substances carried onto the diverse sized fractions of urban particulate matter, especially those under 1 mum in diameter. The mutagenicity of total and sized fractions of urban particulate matter of Bologna (Italy), representative of a medium size town surrounded by small and medium industries, was investigated using the plate incorporation test on Salmonella typhimurium.

There is no correlation between total and/or coarse particle matter concentration in air and mutagenic activity, the correlation increases as the particle size decreases; moreover, the finer the particulate matter, the greater the mutagenicity is. That is of great concern for health risk estimation. Total or inhalable (PM10) particulate matter is not representative of air quality, at least with regard to the mutagenicity.

Parent RA, Caravello HE, San RH. **Mutagenic activity of acrolein in S. typhimurium and E. coli**. J Appl Toxicol 1996;16(2):103-8.
Acrolein was tested for mutagenic activity in seven strains of Salmonella typhimurium and one strain of Escherichia coli using a preincubation assay procedure. Cytotoxicity was evident at dosing levels above 33 and 67 micrograms acrolein per plate in the absence and presence of S-9 activation, respectively. Evidence of mutagenic activity was seen at non-toxic dosing levels in S. typhimurium strains TA98 and TA100 and E. coli strain WP2 uvrA. Responses in TA98 and E. coli were marginal at best, but a firm positive mutagenic activity was noted in TA100 at 20 micrograms per plate without S-9 activation and at 67 micrograms per plate with S-9 activation. The results of this study demonstrate the mutagenicity of acrolein under highly controlled conditions.

Poeller F, Bauch T, Sauerwein W, Boecker W, Wittig A, Streffer C. **Comet assay study of DNA damage and repair of tumor cells following boron neutron capture irradiation with fast d(14) + Be neutrons**. Int J Radiat Biol 1996;70(5):593-602.

Polianskaia GG, Sizova LS. **[The genotoxic effect of ciprofloxacin on cultured cells from the kangaroo rat kidney and on skin fibroblasts from the Indian muntjac]**. Tsitologiia 1996;38(9):958-73. (Rus)
The genotoxicity of an antibiotic ciprofloxacin (CF) in doses of 10, 25, 50 and 100 mkg/ml under its short-term (6-48 h) and long-term (15-30 days) action on sublines of Rat kangaroo kidney.

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 genotoxic response of benzene and tris(2,3-dibromopropyl)-phosphate (TDBP) have been evaluated in several tissues using the standardized lambda/lacI (Big Blue) transgenic mouse mutation assay. Separate groups of four to five male B6C3F1 transgenic lambda/lacI mice were given oral administrations of benzene or TDBP at varying concentrations. Tissues evaluated include lung, bone marrow, and spleen in benzene-treated animals, and liver, kidney, and stomach in TDBP-treated animals. Significant increases in lacI mutations were observed in the spleen and bone marrow of benzene treated mice, and the kidneys of TDBP-treated mice. Where applicable, mutagenesis patterns of tissue sensitivity were consistent with what has been observed previously in other assays. In addition, mutagenicity in tissues not traditionally evaluated for mutations correlated to sites of carcinogenicity for the chemicals tested.

Quillardet P, Touati E, Hofnung M. **Influence of the uvr-dependent nucleotide excision repair on DNA adducts formation and mutagenic spectrum of a potent genotoxic agent: 7-methoxy-2-**

nitronaphtho[2,1-b]furan (R7000). Mutat Res 1996;358(1):113-22.
The influence of the uvr-dependent excision repair system on the lethal action, mutagenic specificity, SOS induction and DNA adducts formation of 7-methoxy-2-nitronaphtho[2,1-b]furan (R7000), a potent genotoxic nitrofuran, were examined in Escherichia coli. Binding measurements of 3H-labelled R7000 to DNA indicated that R7000-DNA adducts can be removed by excision repair soon after the action of the chemical: 50% of the DNA adducts were removed within 10 min of treatment. After 1 h of incubation the level of excision reached 70%. This result was confirmed using the postlabelling technique. We found that R7000 yielded at least 10 different DNA adducts. Each of the adducts detected could be removed by excision repair. The rates of excision appeared different from one to the other. In addition, using a lacZ reversion system that is able to detect each type of base substitution mutations [1], we found that in uvrA bacteria deficient in excision repair, R7000 can induce 5 out of the 6 possible mutational events: GC-->TA, AT-->TA, GC-->CG, AT-->CG and GC-->AT. The transition AT-->GC was not observed. Only 3 transversions: GC-->TA, AT-->TA and GC-->CG could be detected in repair proficient uvr+ bacteria. The differences between the mutagenic spectra obtained in either uvr+ bacteria or uvrA mutants indicate that some potentially mutagenic DNA adducts induced by R7000 can be removed by excision repair, thus lowering the mutagenic potency of the chemical and modifying the mutagenic spectrum detected.

Ransom DG, Haffter P, Odenthal J, Brownlie A, Vogelsang E, Kelsh RN, Brand M, Van Eeden FJ, Furutani-Seiki M, Granato M, et al. **Characterization of zebrafish mutants with defects in embryonic hematopoiesis**. Development 1996;123:311-9.
As part of a large scale chemical mutagenesis screen of the zebrafish (Danio rerio) genome, we have identified 33 mutants with defects in hematopoiesis. Complementation analysis placed 32 of these mutants into 17 complementation groups. The allelism of the remaining 1 blood mutant is currently unresolved. We have categorized these blood mutants into four phenotypic classes based on analyses of whole embryos and isolated blood cells, as well as by in situ hybridization using the hematopoietic transcription factors GATA-1 and GATA-2. Embryos mutant for the gene moonshine have few if any proerythroblasts visible on the day circulation begins and normal erythroid cell differentiation is blocked as determined by staining for hemoglobin and GATA-1 expression. Mutations in five genes, chablis, frascati, merlot, retsina, thunderbird and two possibly unique mutations cause a progressive decrease in the number of blood cells during the first 5 days of development. Mutations in another seven genes, chardonnay, chianti, grenache, sauternes, weiflherbst and zinfandel, and two additional mutations result in hypochromic blood cells which also decrease in number as development proceeds. Several of these mutants have immature cells in the circulation, indicating a block in normal erythroid development. The mutation in zinfandel is dominant, and 2-day old heterozygous carriers fail to express detectable levels of hemoglobin and have decreasing numbers of circulating cells during the first 5 days of development. Mutations in two genes, freixenet and yquem, result in the animals that are photosensitive with autofluorescent blood, similar to that found in the human congenital porphyrias. The collection of mutants presented here represent several steps required for normal erythropoiesis. The analysis of these mutants provides a powerful approach towards defining the molecular mechanisms involved in vertebrate hematopoietic development.

Rao SS, Neheli TA, Carey JH, Herbert A, Hansen PD. **Technical methods section DNA alkaline**

unwinding assay for monitoring the impact of environmental genotoxins. Environ Toxicol Water Qual 1996;11(4):351-4.
BIOSIS COPYRIGHT: BIOL ABS. RRM research article fish mussels toxicology methodology dna alkaline unwinding assay dna environmental genotoxins analytical method.

Riazanova LA, Ptitsyn LR, Ivanov VB, Bystrova EI, Iakovlev KI, Rozhkova ND. **[Comparative evaluation of the genotoxic effect of complex platinum compounds in pro- and eukaryotic test-systems]**. Dokl Akad Nauk 1996;350(2):259-62. (Rus)

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 herbicide trifluralin was evaluated for genotoxicity in cultured human peripheral blood lymphocytes. Sister-chromatid exchanges (SCE), chromosome aberrations (CA) and micronuclei (MN) were scored as genetic endpoints. To detect eventual metabolic modification in the genotoxicity of this herbicide, the cultures for SCE and MN demonstration were also treated with S9 fraction. From our results we can conclude that trifluralin was able to exert a weak cytotoxic effect, reducing both the proliferative rate index (PRI) and the cytokinesis block proliferation index (CBPI), and also to induce a slight but statistically significant increase in the frequency of SCE. Under our conditions of testing, no genotoxic effects of trifluralin were observed in the CA and MN assays.

Salone B, Pretazzoli V, Bosi A, Olivieri G. **Interaction of low-dose irradiation with subsequent mutagenic treatment: role of mitotic delay**. Mutat Res 1996;358(2):155-60.
Experiments were carried out with human lymphocytes to test whether there was any relation between the changes that conditioning treatment can produce in cell progression or in mitotic delay induced by the challenge dose and the presence of an 'adaptive response' (AR). In experiments in which the cells were successively fixed after the challenge dose, the interaction between conditioning treatment and challenge was of the same sign for all the fixation times: therefore it is likely that modifications of the cytogenetic damage in primed cells is not a mere reflection of stage sensitivity. In experiments in which using 1 Gy as conditioning treatment we induced a drastic extension of G2, we did not observe any AR; therefore, even if conditioning treatment can induce modifications in the cell-cycle phases before and/or after challenge, there is probably no link between these modifications and the presence of an AR.

Salonen T, Haapalina A, Heinonen E, Suhonen J, Hervonen A. **Monoamine oxidase B inhibitor selegiline protects young and aged rat peripheral sympathetic neurons against 6-hydroxydopamine-induced neurotoxicity**. Acta Neuropathol (Berl) 1996;91(5):466-74.

Schweikl H, Schmalz G, Gottke C. **Mutagenic activity of various dentine bonding agents**. Biomaterials 1996;17(14):1451-6.
The potential mutagenicity of bonding agents of the new generation was characterised by employing an in vitro gene mutation assay. Eight different components of three dentine bonding systems (Scotchbond Multi Purpose, Prisma Universal Bond 3 and C&B Metabond) were tested in the Ames test using four different Salmonella strains (TA97a, TA98, TA100 and TA102). The materials were eluted in dimethyl

sulphoxide and physiological saline; aliquots of the serially diluted eluates were then used in the standard plate incorporation assay. No mutagenic effects were found with Scotchbond Multi Purpose primer and adhesive.

Sciacchitano CJ, Hirshfield IN. **Molecular detection of Clostridium botulinum type E neurotoxin gene in smoked fish by polymerase chain reaction and capillary electrophoresis**. J AOAC Int 1996;79(4):861-5.
The polymerase chain reaction (PCR), a rapid, sensitive technique for amplifying target DNA sequences of pathogenic microorganisms, was used to amplify Clostridium botulinum type E neurotoxin gene fragments in smoked fish. Other botulinal neurotoxin-producing strains, nontoxigenic strains, and food-related microorganisms did not yield nonspecific amplification products with this PCR assay. PCR products were analyzed by capillary electrophoresis (CE) using a low-viscosity entangled polymer system. Resolution, sensitivity, and DNA sizing accuracy were improved, and analytical times were markedly shortened. The PCR/CE assay detected the C. botulinum type E neurotoxin gene in as few as 10 cells. The technique to other foods may also be a valuable tool for detecting foodborne pathogens.

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.
Big Blue (BB) and generic B6C3F1 mice were given one to three i.p. injections of 50 mg/kg benzo[a] pyrene (B[a]P) in DMSO every other day to achieve cumulative doses of 50 to 150 mg/kg. Three weeks after treatment, the mutation frequency at the endogenous hprt gene and lacI transgene was measured in splenic T cells. Generic mice given 50, 100, and 150 mg/kg B[a]P displayed induced hprt frequencies (observed hprt frequency minus control frequency) of 5.5 +/- 1.0, 11 +/- 2.0, and 19 +/- 2.6 x 10(-6), respectively (average +/- SEM). In contrast, BB mice given 50 and 150 mg/kg B[a]P displayed induced hprt frequencies of 0.9 +/- 0.6 and 9.1 +/- 1.5 x 10(-6). 32P postlabelling revealed that the lower hprt response in BB mice correlated with lower amounts of BP-DNA adducts in spleen, liver, and lung 24 hours after B[a]P exposure. Western blot analysis of liver samples from B[a]P-treated mice suggests that the reduced adduct load in turn may be due to lower P450 1A1 levels in BB mice. The frequency of induced, nonsectored blue plaques (observed blue plaque frequency minus control frequency) in BB mice receiving 50 and 150 mg/kg B[a]P was 41 +/- 9 and 134 +/- 10 x 10(-6) (15- to 40-fold higher than the induced hprt frequency in the same treated animals). Sectored plaques were observed in both control and B[a]P groups but their frequency showed no relationship to dose (sectored frequency in all groups was approximately 20 x 10(-6)). To test whether persistent DNA adducts in the packaged lambda vector were contributing to the observed blue plaque frequency, purified lambda-LIZ DNA was treated in vitro with B[a]P diol epoxide (BPDE), packaged, and plated on E. coli lawn cells. Treatment with BPDE did not produce significant increases in homogeneous blue plaques, suggesting that the majority of mutants obtained from B[a]P-treated BB mice occurred in vivo. These results indicate that B[a]P exposure produces many more mutations at the lacI transgene than at the endogenous hprt locus.

Speit G, Hanelt S, Helbig R, Seidel A, Hartmann A. **Detection of DNA effects in human cells with the comet assay and their relevance for mutagenesis**. Toxicol Lett 1996;88(1-3):91-8.
The single cell gel test (SCG-test or comet assay) is a rapid and sensitive method for measuring DNA damage and repair in individual cells. A wide variety of mutagens have been shown to cause DNA

alterations detectable with the comet assay, but it is not yet clear whether a relationship exists between the DNA effects and the induction of mutations. We are therefore investigating in a cell culture system with human cells (MRC5CV1) the induction of DNA damage by environmental mutagens and the formation of mutations at the HPRT gene. In the present study we investigated benzo[a]pyrene (BP), an environmental mutagenic and formation of mutations at the HPRT gene. In the present study we investigated benzo[a]pyrene (BP), an environmental mutagenic and carcinogenic polycyclic aromatic hydrocarbon, and its reactive metabolite (+)-anti-benzo[a]pyrene-7,8-diol 9, 10-oxide ((+)-anti-BPDE). S9 mix activated BP and the direct acting mutagen (+)-anti-BPDE caused a concentration-related increase in DNA migration in the comet assay. Postincubation experiments indicated that induced DNA effects are eliminated by DNA repair within 24 h. BP-treatment caused a strong genotoxic effect in the comet assay but had only a marginal effect on the frequency of gene mutations. When cells were treated with BP in the presence of cadmium sulphate, a clear increase in genotoxicity was observed while the effect on mutations was unchanged. Our results indicate that DNA alterations detected with the comet assay do not necessarily relate to mutagenesis. The absence of a close relationship between DNA migration in the comet assay and mutagenesis may be explained by the fact that some effects seen in the comet assay occur as a consequence of an error free DNA repair process.

Staleva L, Waltscheva L, Golovinsky E, Venkov P. **Enhanced cell permeability increases the sensitivity of a yeast test for mutagens**. Mutat Res 1996;370(2):81-9.
BIOSIS COPYRIGHT: BIOL ABS. ts1 is a mutation which causes a general increase in permeability of Saccharomyces cerevisiae cells in an unspecific manner. The introduction of the ts1 mutation under homozygous conditions into the D7 diploid strain enhanced the sensitivity of the test system described by Zimmermann et al. (1975). The newly constructed strain D7ts1 responded with a four to six times higher frequency compared to the D7 strain for all genetic end-points induced with chemical mutagens (ethyl methanesulfonate, methyl methanesulfonate, hydroxyurea, benzpyrene). The increased sensitivity of D7ts1 is specific only for mutagens active in yeast, since treatment of D7ts1 cells with 5-bromouracil or 5-bromouridine, known to be non-mutagenic in yeast, did not result in the induction of any of the measured genetic alterations. Five out of 14 water samples taken from the environment induced recombinogenic events in D7ts1, whereas all 14 water samples were without effect in the D7 test system. We concluded that D7ts1 cells show a higher sensitivity in the detection of mutagenic or carcinogenic action because of their generally enhanced permeability due to the ts1 mutation.

Stallard N, Whitehead A. **The fixed-dose procedure and the acute-toxic-class method: a mathematical comparison**. Hum Exp Toxicol 1995;14(12):974-90.
The fixed-dose procedure (FDP), proposed by the British Toxicology Society, and the acute-toxic-class (ATC) method, proposed by the German Federal Health Authority, provide alternatives to the LD50 test for classifying substances by their acute oral toxicity. This paper presents a mathematical model that is used to compare the two procedures in terms of their classification properties and the required numbers of animals. It is found that the classification properties of the procedures depend on the dose levels used, the number of animals tested per dose and the criteria that are used to decide whether testing should continue at a higher or lower dose. For substances with steep dose-response curves, the most likely classification is determined chiefly by the choice of the dose levels whilst the number of animals and continuation criteria used are increasingly important for substances with dose-response curves with a

smaller slope. The use of toxicity as a possible endpoint as in the FDP and the use of a two-stage testing procedure at each dose as in the ATC method are both found to reduce the expected numbers of animals required with little effect on the classification properties. On the strength of these findings it is indicated that a new procedure combining the dose levels and testing approach of the ATC method with the inclusion of toxicity as an endpoint as in the FDP would be more efficient than either the FDP or the ATC method.

Suter W, Ahiabor R, Blanco B, Locher F, Mantovani F, Robinson M, Sreenan G, Staedtler F, Swingler T, Vignutelli A, et al. **Evaluation of the in vivo genotoxic potential of three carcinogenic aromatic amines using the Big Blue transgenic mouse mutation assay**. Environ Mol Mutagen 1996;28(4):354-62.
Three genotoxic mouse carcinogens, 4-chloro-o-phenylenediamine (4-C-o-PDA), 2-nitro-p-phenylenediamine (2-N-p-PDA), and 2,4-diaminotoluene (2,4-DAT), were tested in the Big Blue transgenic mouse mutation assay. Each experiment consisted of a vehicle control group with ten Big Blue C57BL/6 mice, five of either sex, and an equally sized group treated with a high dose of the test chemical. In addition, four animals were treated with the vehicle and six animals with the test compound for the measurement of bromodeoxyuridine (BrdU) incorporation to determine cellular proliferation. Prior to the mutagenicity experiments, the maximally tolerated dose of each compound was determined using nontransgenic C57BL/6 mice. Based on these results the doses used in the main study were 200 mg/kg/day for 4-C-o-PDA, 150 mg/kg/ day for 2-N-p-PDA, and 80 mg/kg/day for 2,4-DAT. Animals were treated for 10 days over a 2 week period and were killed 10 days after the ast treatment. In an additional experiment with 2,4-DAT, animals were killed 28 days after treatment. Since all three chemicals are liver carcinogens in the mouse, the DNA of the liver was analyzed using the standard procedures for the Big Blue assay. Hepatocyte proliferation was assessed by immunohistochemical detection of proliferating cell nuclear antigen (PCNA) and, in some studies, by measuring BrdU.

Tafazoli M, Kirsch-Volders M. **In vitro mutagenicity and genotoxicity study of 1,2-dichloroethylene, 1,1,2-trichloroethane, 1,3-dichloropropane, 1,2,3-trichloropropane and 1,1,3-trichloropropene, using the micronucleus test and the alkaline single cell gel electrophoresis technique (comet assay) in human lymphocytes**. Mutat Res 1996;371(3-4):185-202.

Tichy M, Rucki M. **[Alternative method for the determination of acute toxicity of chemicals: inhibition of movements of the worms Tubifex tubifex]**. Prac Lek 1996;48(6):225-30. (Cze)
BIOSIS COPYRIGHT: BIOL ABS. In order to develop a rapid and cheap testing of acute toxicity of compounds soluble in water, the authors selected the determination of effective concentration for the inhibition of movements of the worms Tubifex tubifex and their lethal concentration to the same worms, respectively. The method of the determination has been described and an example of its use is presented. For the inner control of the correctness of determination (QA), the authors suggest to use a solution of manganese chloride dehydrate, whose acute toxicity indices have been determined in a long-term observation: log EC50 = -0.845 : 0.0326 (mol/l) log LC50 = -0.726 : 0.0390 (mol/l) The determination of EC50, LC50, NOEL, EC100 for one chemical lasts about 3 hours including preparation of the solutions and a verification of their concentration, a pilot testing and triplicate determination of toxicity of the tested and reference chemicals.

Tinwell H, Paton D, Guttenplan JB, Ashby J. **Unexpected genetic toxicity to rodents of the N',N'-dimethyl analogues of MNU and ENU**. Environ Mol Mutagen 1996;27(3):202-10.
In an effort to explain the carcinogenic activities of N',N'-dimethyl-N-ethyl-N-nitrosourea (DMENU) and trimethylnitrosourea (3475636) (TMNU) despite their hydrolytic stability, a study was conducted examining the rates of hydrolysis, relative alkylating potential, relative mutagenicity, and relative micronucleus inducing potential of DMENU and TMNU. In contrast to the activity of the parent compounds, N-methyl-N-nitrosourea (684935) (MNU) and N-ethyl-N-nitrosourea (759739) (ENU), neither TMNU nor DMENU had alkylating potential. Examination of the half lives of ENU and MNU suggested that the addition of methyl groups to these compounds greatly diminished, but did not abolish, the rate of hydrolysis. MNU and ENU were potent mutagens in the Salmonella mutagenicity assay and induced dose related increases in the frequency of micronucleated polychromatic erythrocytes. DMENU and TMNU demonstrated weak mutagenic activity in Salmonella-typhimurium (TA-1535) and only at dose levels at which the parent compounds were toxic. The mutagenic activity of DMENU and TMNU was slightly increased with the addition of S9 mix. In the bone marrow micronucleus assay in male CBA-mice, CD-1-mice, or AP-mice, DMENU and TMNU induced similar frequencies of micronuclei as their parent compounds and DMENU induced a stronger response when the dose was adjusted to be equivalent in molarity to that of the parent compound. The dimethyl analogs were less toxic to bone marrow cells compared with the parent compounds. Possible reasons for these findings were discussed.

Tinwell H, Yendle J, Ashby J. **Mutagenicity to the mouse bone marrow by the mouse germ cell mutagen N-propyl-N-nitrosourea**. Mutat Res 1996;370(3-4):141-3.
N-Propyl-N-nitrosourea (PNU) is shown to be active in male mouse bone marrow micronucleus assays when dosed at either 100 or 200 mg/kg in saline. Activity was observed following either intraperitoneal (i.p.) injection or oral gavage. This observation is consistent with the demonstration by Murota and Shibuya of the specific-locus mutagenicity caused by PNU in male mouse spermatogonia when dosed at 200 mg/kg by i.p. injection. These data strengthen further the observation that rodent germ cell mutagens are also mutagenic to rodent somatic cells.

Tomicic M, Franekic J. **Modulation of genotoxic response in Salmonella typhimurium by the overexpression of Escherichia coli 3-methyladenine-DNA glycosylase II (AlkA)**. Period Biol 1996; 98(3):395-8.
BIOSIS COPYRIGHT: BIOL ABS. Background and purpose: Salmonella typhimurium does not harbour an inducible glycosylase activity, and responds very weakly to alkylation-induced damage. We wanted to investigate in vivo effects of the overexpressed E. coli glycosylase II activity on survival capacity and mutation induction in S. typhimurium, and thereby to elucidate the significance of both 3-methylpurines in cellular sensitivity to methylating agents. Material and methods: HisG46 and TA1535 strains of S. typhimurium were transformed with the pMT2 plasmid, carrying the alkA gene under the control of the lac promoter. The overexpression of the AlkA protein was measured in the glycosylase activity assay. Resistance of transformants to cytotoxic and mutagenic effects of dimethyl sulfate was established in the survival and Ames mutation induction assay. Results: Although the 3-methyladenine-DNA glycosylase II activity in bacterial extracts of the TA1535/pMT2 transformants was about 130-fold higher on exposure to IPTG compared to the TA1535/pUC8 control, the overexpression of the AlkA protein could not completely suppress AlkA- phenotype of S. typhimurium in neither of the

experimental strains. However, the TA1535/pMT2 transformants, deficient in the nucleotide excision repair (uvrB-), showed about 20% survival increase compared to the transformants of hisG46, which are uvrB+. The yield of mutation induction in the reversion assay was decreased to the level of spontaneous revertant (his- - his+) colonies. Conclusions: The cleavage of methylated purines is interfered with the UvrABC enzyme. In overexpressed conditions in vivo, 3-methylguanine, in addition to 3-methyladenine, must have been removed from DNA by the E. coli AlkA protein, and therefore accounts not only for the second most important cytotoxic, but also for a potential mutagenic lesion.

Tronov VA, Pelevina II. **[The DNA-comet method for individual cells. The principle and use of the method]**. Tsitologiia 1996;38(4-5):427-39. (Rus)
The DNA comet assay allows to evaluate the damage in genomes of individual cells. This article summaries the literary evidence and the author's experience in development and application of the technique. Principles of the method are reviewed in addition to protocols for neutral and alkaline conditions; examples of application of the method are given.

Van Berkel PH, Van Veen HA, Geerts ME, De Boer HA, Nuijens JH. **Heterogeneity in utilization of N-glycosylation sites Asn624 and Asn138 in human lactoferrin: a study with glycosylation-site mutants**. Biochem J 1996;319(Pt 1):117-22.
Human lactoferrin (hLF) is a glycoprotein involved in the host defence against infection and excessive inflammation. Our objective was to determine to what extent each of the three sequons for N-linked glycosylation in hLF is actually used. Human kidney-derived 293(S) cell lines expressing recombinant hLF (rhLF) or glycosylation-site mutants were produced. The mutations involved replacement of asparagine residues with glutamine at one or more sequons for N-glycosylation (Asn138, Asn479 and Asn624).

Vitvitskii VN, Bakhitova LM, Soboleva LS, Shevchenko VA. **[Modification of the mutagenic effects of gamma radiation by heavy metal salts]**. Izv Akad Nauk Ser Biol 1996;(4):495-8. (Rus)
The effect of K2Cr2O7 and (CH3COO)2Pb on the mutagenic activity of gamma rays was studied by a micronucleus test in mouse bone marrow polychromatocytes. Acute and chronic combined actions of the two factors were investigated. Chromium ions (VI) enhanced mutagenic effects of gamma rays in both acute and chronic.

Vrzoc M, Petras M. **Comparison of three power supplies used for the single-cell gel assay**. Environ Mol Mutagen 1996;28(2):154-7.
BIOSIS COPYRIGHT: BIOL ABS. The alkaline single-cell gel (SCG) assay, which determines DNA damage in mice treated with 100 mg/kg methyl methanesulfonate (MMS), was run by using three power supplies from Bio-Rad (models 3000Xi, 200/2.0, and Power Pac 300). Comparisons of the results obtained from the use of these power supplies showed differences in mean DNA tail length to width ratios and profiles of these ratios in individual calls. Model 200/2.0 power supply appeared to enhance significantly the sensitivity of the assay. In purchasing power supplies, there is a need to evaluate their effect on the sensitivity of the test.

Wagner ED, Cebulska-Wasilewska A, Connolly S, Plewa MJ. **Mutagenic analysis of 2,3-diaminophenazine and 2-amino-3-hydroxyphenazine in Salmonella strains expressing different**

levels of O-acetyltransferase with and without plant and mammalian activation. Mutat Res 1996;372(1):65-74.
2,3-Diaminophenazine (DAP) and 2-amino-3-hydroxyphenazine (AHP) are products generated from oxidative-type phenylenediamine hair dyes and are also present in pesticide formulations as contaminants. Earlier studies demonstrated that DAP and AHP were mutagenic in Salmonella typhimurium strains after mammalian microsomal activation. Plant systems can activate structurally similar arylamines. S. typhimurium strains have been developed that express elevated levels of acetyl-CoA: N-hydroxyarylamine O-acetyltransferase (OAT). O-acetyltransferase expression is necessary for the generation of the ultimate arylamine promutagen after plant activation. A number of arylamines including 2-aminofluorene, benzidine and 4-aminobiphenyl were activated by plant cells into mutagens in the OAT over-expressing S. typhimurium strain, YG1024. The objectives of this research were to examine the mutagenicity of DAP and AHP with mammalian or plant activation in Salmonella strains with different acetyltransferase activities. The hypothesis tested was whether and to what degree a metabolite of DAP or AHP could serve as a substrate for bacterial O-acetyltransferase and induce mutation in Salmonella. DAP and AHP without activation induced both frameshift and base pair substitution mutations in S. typhimurium strains that exhibited elevated levels of O-acetyltransferase activity. The mutagenicity of DAP and AHP were greatly enhanced with mammalian hepatic microsomal activation resulting in a preferential induction of frameshift mutations. With the hisD3052 allele as the gene target, S9-activated DAP induced frameshift mutations in YG1024 and TA98 as well as the OAT deficient strain.

Waldmann P. **[Alkaline filter elution as a method for the demonstration of genotoxic potentials in surface waters]**. Munch Beitr Abwasser Fisch Flussbiol 1996;49:391-402. (Ger)
BIOSIS COPYRIGHT: BIOL ABS. RRM book chapter leuciscus-idus corbicula-fluminea mussel alkaline filter elution surface waters toxicology methodology genotoxicity testing method genotoxic potential rhine river main river germany.

Wang X, Kitamura K, Yamamoto K. **Mutagenic specificity of N-methyl-N'-nitro-N-nitrosoguanidine in the tonB gene on the chromosome of Escherichia coli recA+ and recA- cells**. Biochem Biophys Res Commun 1996;227(2):334-9.
DNA base sequence changes induced by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) mutagenesis has been determined for the endogenous tonB gene of Escherichia coli recA+ strain and its isogenic recA56 strain. In the recA+ strain, base substitutions accounted for 48 mutations among 54 MNNG-induced independent mutations analyzed and consisted of 45 G:C to A:T transitions, two A:T to G:C transitions and one A:T to C:G transversion. In the recA56 strain, 67% (34/51) were base substitutions among which G:C to A:T transition (28/51) predominated, followed by three A:T to T:A transversions, two G:C to T:A transversions, and one A:T to G:C transition. These mutagenic specificities were consistent with the mispairing predicted by the methylation of the O6 position of guanine. In both strains the G:C to A:T mutations were found at guanine residues preceded by either guanine or thymine on the non-transcribed strand of the target gene.

Weisburger JH, Hara Y, Dolan L, Luo FQ, Pittman B, Zang E. **Tea polyphenols as inhibitors of mutagenicity of major classes of carcinogens**. Mutat Res 1996;371(1-2):57-63.
Previous research suggested that the mutagenicity of some genotoxic carcinogens, mainly heterocyclic

amines, was decreased by green or black tea extracts, or tea polyphenol fractions. Thus, it seemed important to test a variety of genotoxic carcinogens with distinct chemical structures and means of biochemical activation as regards modification of mutagenicity in appropriate strains of Salmonella typhimurium by 3 concentrations of polyphenols 60, 100, or B, standard commercial polyphenol preparations from green or black tea. Polyphenols sharply decreased the mutagenicity of a number of aryl- and heterocyclic amines, of aflatoxin B1, benzo[a]pyrene, 1,2-dibromoethane, and more selectively, of 2-nitropropane, all involving an induced rat liver S9 fraction. Good inhibition was found with 2 nitrosamines that required a hamster S9 fraction for biochemical activation. No effect was found with 1-nitropyrene, and with the direct-acting (no S9) 2-chloro-4-methyl-thiobutanoic acid. Thus, with some exceptions, polyphenols considerably decreased the mutagenicity of diverse types of carcinogens.

Williams GM, Aardema MJ, Long PH, Thompson ED, Allgood GS. **Genotoxicity and subchronic toxicity studies with heated olestra**. Food Chem Toxicol 1996;34(10):941-50.
Olestra is a class of sucrose-fatty acid polyesters intended for use as a non-caloric replacement of edible oil. Genotoxicity and subchronic toxicity studies were conducted to determine whether olestra could form genotoxic or toxic breakdown products during simulated commercial use. Heated olestra was prepared for these studies by batch-frying potato slices in olestra at 177-185 degrees C for 25-32 hr over 5-7 days. Genotoxicity of this previously heated olestra was assessed in four standard in vitro assays: (1) Salmonella mutagenesis (Ames test); (2) forward mutagenesis of mouse lymphoma cells at the thymidine kinase locus; (3) unscheduled DNA synthesis in rat hepatocytes; and (4) clastogenicity in cultured Chinese hamster ovary cells. These tests were conducted with previously heated olestra at concentrations up to at least 5 mg/ml both in the absence of exogenous bioactivation and, for assays (1), (2) and (4) with added liver microsomal (S-9) activation. The Ames and mouse lymphoma assays were performed with olestra (10 mg/ml and 23 mg/litre, respectively) either alone or emulsified with the non-toxic, non-ionic surfactant Pluronics F68, both in the presence and absence of metabolic activation. To test for clastogenicity in vivo, rats were administered previously heated olestra by gavage at 5 g/kg per day for up to 5 days and bone marrow cells were examined for chromosomal aberrations. Heated olestra lacked genotoxic activity detectable by the aforementioned assays. Heated olestra was fed to Fischer 344 rats at up to 10% of the diet (w/w) for 91 days. Evaluation of survival, food consumption, feed efficiency, physical condition, body weight, organ weight, haematological and clinical chemistry parameters, and histomorphology revealed no adverse effects attributable to ingestion of heated olestra at exposure levels in excess of those anticipated for human consumption. It is concluded that olestra used as a deep-frying medium conveys no genotoxic or toxic hazard at anticipated levels of human consumption.

Woods AC, Guillemette JG, Parrish JC, Smith M, Wallace CJ. **Synergy in protein engineering. Mutagenic manipulation of protein structure to simplify semisynthesis**. J Biol Chem 1996;271(50): 32008-15.
Semisynthesis is a chemical technique of protein engineering that provides a valuable complement to directed mutagenesis. It is the method of choice when the structural modification requires, for example, a noncoded amino acid. The process involves specific and limited protein fragmentation, structural manipulation of the target sequence, and subsequent religation of fragments to give the mutant holoprotein. We suggested and demonstrated that mutagenesis and semisynthesis could be used

synergistically to achieve protein engineering goals otherwise unobtainable, if mutagenesis was used to shuffle methionine residues in the yeast cytochrome c sequence (Wallace, C. J. A., Guillemette, J. G., Hibiya, Y., and Smith, M. (1991) J. Biol. Chem. 266, 21355-21357). These residues can not only be sites of specific cleavage by CNBr but also of spontaneous peptide bond synthesis between fragments in noncovalent complexes, which greatly facilitates the semisynthetic process. We have now used an informed methionine scan of the protein sequence to discover other useful sites and to characterize the factors that promote this extraordinary and convenient autocatalytic religation. Of eight sites canvassed, in a wide range of settings, five efficiently provoked peptide bond synthesis. The principal factor determining efficiency seems to be the hydropathy of the religation site. The mutants created have also provided some new insights on structure-function relationships in the cytochrome.

Woudstra EC, Roesink JM, Rosemann M, Brunsting JF, Driessen C, Orta T, Konings AW, Peacock JH, Kampinga HH. **Chromatin structure and cellular radiosensitivity: a comparison of two human tumour cell lines**. Int J Radiat Biol 1996;70(6):693-703.
The role of variation in susceptibility to DNA damage induction was studied as a determinant for cellular radiosensitivity. Comparison of the radiosensitive HX142 and radioresistant RT112 cell lines previously revealed higher susceptibility to X-ray-induced DNA damage in the sensitive cell line using non-denaturing elution, but not when using alkaline unwinding. The present data also show that no difference in the amount of initial damage is seen when pulsed-field gel electrophoresis (PFGE) or comet analysis are used for DNA damage assessment. However, using the halo assay or a modified version of PFGE in which the higher DNA architecture remained partially intact, the radiosensitive cells showed steeper dose-response curves for initial DNA damage than the radioresistant cells. Analysis of the protein composition, of DNA-nucleoid structures revealed substantial differences when isolated from HX142 or RT112 cells. From our data, it is concluded that HX142 and RT112 differ in their structural organization of chromatin. As no differences in the kinetics of DNA damage rejoining were found, it is hypothesized that the same amount of lesions have a different impact in the two cell lines in that the 'presentation' of DNA.

Wu X, Hsu TC, Spitz MR. **Mutagen sensitivity exhibits a dose-response relationship in case-control studies**. Cancer Epidemiol Biomarkers Prev 1996;5(7):577-8.
We have been quantitating, as a marker of cancer susceptibility, induced chromatid breaks in lymphocyte cultures exposed to chemical mutagens. This report highlights the consistency of the results from two case-control studies, using different methods of presenting the data. In both the lung cancer case-control study, which used bleomycin, a radiomimetic agent, as the test mutagen, and the melanoma study, which used 4-nitroquinoline-oxide, an UV-mimetic agent, the mean number of breaks/cell was significantly higher in the cases compared with the controls. When the data were dichotomized at the 75th percentile of breaks in the control populations, significantly elevated adjusted odds ratios (3.7 and 5.0, respectively) were detected. Dose-response relationships were evident in both studies when the data were categorized by quartiles of breaks/cell in the controls, with highest risk estimates being in the top quartile of induced breaks. The potential for extending this assay to other cancer sites, using a variety of test mutagens, is exciting.

Yoshikawa K, Inagaki K, Terashita T, Shishiyama J, Shankel DM. **Desmutagenic effect of pheophytin from Japanese eggplant against several mutagens**. J Food Hyg Soc Jpn 1996;37(5):295-300.

BIOSIS COPYRIGHT: BIOL ABS. Using Salmonella typhimurium TA98 in the Ames test, we have identified desmutagens in Japanese eggplant juice. Most of the desmutagenic activity in the eggplant fruit is due to pheophytin, a Mg-free derivative of chlorophyll. Pheophytin inhibits mutagenesis to about 40% with or without metabolic activation of mutagens by S9 mix preparations. The mutagens employed were 2-aminoanthracene (2-AA), 2-aminofluorene (2-AF), 2-amino-3-methyl-9H-pyrido(2,3-b)indole (MeAalphaC), 3-amino-1-methyl-5H-pyrido(4,3-b)indole (Trp-P-2) and 2-nitrofluorene (2-NF). Pheophytin did not influence enzymes involved in metabolic activation or interfere with the activation process when S9 was added. Therefore pheophytin appears to be a desmutagen acting directly on the mutagens. Trp-P-2-pheophytin reaction products could not be isolated by HPLC or determined by 1H-NMR under various conditions owing to their instability. The desmutagenicity of pheophorbide, the phorbin skeleton of pheophytin, against Trp-P-2 was approximately 40%, but phytol, the carbon chain of pheophorbide, showed no desmutagenicity. This suggests that the desmutagenicity of pheophytin is derived from the pyrole rings.

Yuan ZM, Huang Y, Fan MM, Sawyers C, Kharbanda S, Kufe D. **Genotoxic drugs induce interaction of the c-Abl tyrosine kinase and the tumor suppressor protein p53**. J Biol Chem 1996;271 (43):26457-60.
The function of the c-Abl protein tyrosine kinase is unknown. The present studies demonstrate that the antimetabolite 1-beta-D-arabinofuranosylcytosine (ara-C) induces binding of c-Abl and p53. Ara-C treatment of cells that express wild type or a dominant negative, kinase-inactive c-Abl(K-R) was associated with formation of c-Abl-p53 complexes and increased expression of the cyclin-dependent kinase (Cdk) inhibitor p21. However, down-regulation of Cdk2 by ara-C was found in cells expressing wild type c-Abl and not in cells expressing c-Abl(K-R) or those deficient in p53. Similar findings were obtained following treatment of cells with the alkylating agent methyl methanesulfonate (MMS). Cells that express the c-Abl dominant negative or are null for c-Abl exhibited partial abrogation of Cdk2 down-regulation and G1 arrest in response to MMS exposure. Cells lacking the c-abl gene also responded to ara-C and MMS with increases in p53 levels and induction of p21. These findings indicate that the cellular response to certain genotoxic drugs involves binding of c-Abl to p53 and down-regulation of Cdk2 by a c-Abl kinase/p53-dependent mechanism.

Zahn RK. **[Detection and evaluation of genotoxic risks]**. Munch Beitr Abwasser Fisch Flussbiol 1996;49:364-78. (Ger)
BIOSIS COPYRIGHT: BIOL ABS. RRM book chapter human rat mouse polycyclic aromatic hydrocarbons benzo a pyrene trichloroethylene polybrominated biphenyls dichloromethane carbon tetrachloride chloroform drinking water genotoxic risks toxicology detection evaluation.

Zardoya R, Abouheif E, Meyer A. **Evolutionary analyses of hedgehog and Hoxd-10 genes in fish species closely related to the zebrafish**. Proc Natl Acad Sci U S A 1996;93(23):13036-41.
CBAC COPYRIGHT: CHEM ABS The study of development has relied primarily on the isolation of mutations in genes with specific functions in development and on the comparison of their expression patterns in normal and mutant phenotypes. Comparative evolutionary analyses can complement these approaches. Phylogenetic analyses of Sonic hedgehog (Shh) and Hoxd-10 genes from 18 cyprinid fish species closely related to the zebrafish provide novel insights into the functional constraints acting on Shh. Our results confirm and extend those gained from expression and cryst. structure analyses of this

gene. Unexpectedly, exon 1 of Shh is found to be almost invariant even in third codon positions among these morphol. divergent species suggesting that this exon encodes for a functionally important domain of the hedgehog protein. This is surprising because the main functional domain of Shh had been thought to be that encoded by exon 2. Comparisons of Shh and Hoxd-10 gene sequences and of resulting gene trees document higher evolutionary constraints on the former than on the latter. This might be indicative of more general evolutionary patterns in networks of developmental regulatory genes interacting in a hierarchical fashion. The presence of four members of the hedgehog gene family in cyprinid fishes was documented and their homologies to known hedgehog genes in other vertebrates were established.

Zhuang ZX, Shen Y, Shen HM, Ng V, Ong CN. **DNA strand breaks and poly (ADP-ribose) polymerase activation induced by crystalline nickel subsulfide in MRC-5 lung fibroblast cells**. Hum Exper Toxicol 1996;15(11):891-7.
BIOSIS COPYRIGHT: BIOL ABS. Nickel compounds are potent carcinogens. Their carcinogenicity is believed to be associated with their solubility and cellular uptake. In the present study, we assessed the in vitro genotoxic effect of a water-insoluble nickel compound, crystalline nickel subsulfide (alpha-Ni3S2) on human embryo lung fibroblast cell line (MRC-5 cells). DNA strand breaks was evaluated using single cell gel electrophoresis, or comet assay. The alpha-Ni3S2 induction of poly (ADP-ribose) polymerase (PADPRP), a nuclear enzyme associated with DNA damage and repair was also studied. Hydrogen peroxide (H2O2) was used as a reference compound. A dose-response relationship was found between alpha-Ni3S2 concentrations (2.5 mug/cm2 to 20 mug/cm2) and the comet tail length. The increase of PADPRP activity of alpha-Ni3S2 treated MRC-5 cells was also significant and dose-dependent within the concentration range of 2.5 mug/ cm2 to 10 mug/cm2. Close associations have been found between the comet length and PADPRP level for H2O2 (r=0.98) and alpha-Ni3S2 (r=0.97). These results clearly suggest that alpha-Ni3S2 is a potent agent in inducing DNA strand breaks, which may be closely related to its carcinogenic effects. Data from the present study also suggest that both comet assay and PADPRP determination are sensitive techniques for quantitative evaluation of DNA damage induced by nickel compounds.

HEPATIC AND RENAL TOXICITY

Boot JH. **Hepatotoxic effects of SH-reagents in human and rat hepatocyte cultures and in situ perfused rat livers**. Cell Struct Funct 1996;21(4):221-9. Effects organic mercurials (PCMBS, PCMB, mersalyl) an alkylating reagent (NEM), disulphide reagents (DTP, CPDS) and the dithiocarbamate agent DSF (disulfiram) were studied in hepatocyte culture. Cytotoxicity, was on a high level (organic mercurials), moderate (NEM, DTP), or none (DSF, CPDS). The organic mercurials and NEM induced glutathione depletion. Disulphide compounds were detoxified by metallothionein binding. Organic mercurials inhibited the cellular glucose uptake. The most prominent effect of NEM, DTP and DSF was an inhibition of the TCA-cycle. The hepatocellular BSP metabolism was delayed by all tested compounds. Albumin synthesis was stimulated by pyruvate and blocked by PCMB and PCMBS, by inhibiting the hepatocellular amino acid uptake. Phase I and II biotransformation reactions were inhibited by PCMBS and PCMB by direct binding to Cyt. P450 cysteinyl-residues and active sites of UDP-glucuronyltransferases. DSF probably reacts by diminishing the availability of the cofactor

NADPH. Isolated ALDH (EC 1, 2, 1.3) was inhibited by all studied compounds. In cellular systems, DSF and the organomercurials inhibited ALDH, thereby reducing the cell's capacity of ethanol catabolism. All tested compounds showed, in low doses, the anabolic ability of insulin mimicking, as demonstrated in a balanced endocrine in vitro testsystem. Morphology, Exposure to NEM, DTP, CPDS, DSF did not result in any morphological alterations in the cell cultures. However, an exposure to PCMBS and PCMB, resulted in extensive bleb-formation, as a result of SH group blocking at the cell's outer membrane. It can be concluded, that cultured hepatocytes from human or rat origin, resist an exposure to alkylating and disulphide SH-reagents up to relatively high dose (1.0 mM). However, organic mercury compounds triggered an extensive bleb formation, as a result of SH-blocking, thereby disturbing the osmotic balance by blocking Na+/K+ carriers. Of all tested reagents, organic mercury compounds arose as the most toxic reagents.

Chemin I, Takahashi S, Belloc C, Lang MA, Ando K, Guidotti LG, Chisari FV, Wild CP. **Differential induction of carcinogen metabolizing enzymes in a transgenic mouse model of fulminant hepatitis**. Hepatology 1996;24(3):649-56.
The objective of this work is to examine the possible modulation of carcinogen metabolism (activation by cytochrome P450s and detoxification by conjugation via glutathione S-transferases [GST]) in relation to hepatitis B virus (HBV)-associated liver injury. In HBV transgenic mouse lineage 107.5, the hepatitis B surface antigen (HBsAg) is expressed at noncytopathic concentrations but after injection of an HBsAg-specific, major histocompatibility complex (MHC) class I restricted cytotoxic T-lymphocyte (CTL) clone, the mice develop a severe acute necroinflammatory liver disease that reaches maximum severity within 3 days and gradually subsides during the next 2 to 3 weeks. In this model, using immunohistochemical analysis, we observed an increase of P450s (CYP1A and 2A5), both involved in aflatoxin B1, metabolism, but minor changes or no changes for others (2B, 2C, 2E, 3A). There was a fivefold decrease in the total liver P450 microsomal content 3 days' post-CTL injection with the result that the relative proportion of CYP2A5 and 1A compared with other P450s is increased. Individual microsomal P450 enzyme contents estimated by Western blotting; Northern blot analysis of liver CYP messenger RNA (mRNA) levels as well as in vitro metabolism of specific substrates for different P450 isoenzymes were consistent with the immunohistochemical data. Immunohistochemical staining with antibodies to cytosolic pi class GST was increased 1 and 3 days postinjection followed by a progressive decrease at later time points (the same phenomenon was observed to a lesser extent for GST alpha). The activity of hepatic cytosols toward substrates specific for different subclasses of GST (mu, pi, alpha) showed that while GST mu was not changed in the CTL-injected HBV transgenic mice, GST pi and, to a lesser extent, alpha were increased as compared with controls. These results suggest that liver cell injury induced by a process of acute fulminant-like hepatitis can lead to the induction of some carcinogen metabolizing enzymes notably, Cyp 1A, 2A5 and GST pi in the mouse.

Cheng CC, Etoh J, Tanimura T, Egashira Y, Ohta T, Sanada H. **Effects of dietary gluten on the hepatotoxic action of galactosamine and/or endotoxin in rats**. Biosci Biotechnol Biochem 1996;60 (3):439-43.
This study was done to clarify the effects of dietary wheat gluten on the hepatotoxic action of D-galactosamine (GalN) and endotoxin (Etx). Male Wistar rats fed a high casein or high gluten (supplemented with L-Lys and L-Thr) diet were injected with GalN or Etx, and the plasma glutamate

oxaloacetate transaminase, glutamate pyruvate transaminase, and lactase dehydrogenase activities were examined 20 h later. In rats fed the high gluten diet, these enzyme activities were lower than in the high casein group after injection of 800 mg/kg of GalN. But such a difference between the casein and gluten groups was not clear when they were treated with 400 mg/kg of GalN nor observed even after injection of Etx or Etx+GalN (400 mg/kg). Similarly these was no difference in the plasma concentrations of Etx, tumor necrosis factor-alpha, or interferon-gamma in the rats receiving an injection of 800 mg/kg of GalN between both dietary groups. These results suggest that dietary gluten affords protection against hepatic injury by a high dose of GalN but not by a low dose of GalN and/or Etx.

Drahushuk AT, McGarrigle BP, Tai HL, Kitareewan S, Goldstein JA, Olson JR. **Validation of precision-cut liver slices in dynamic organ culture as an in vitro model for studying CYP1A1 and CYP1A2 induction**. Toxicol Appl Pharmacol 1996;140(2):393-403.

Ring JA, Ghabrial H, Ching MS, Shulkes A, Smallwood RA, Morgan DJ. **Conjugation of p-nitrophenol by isolated perfused fetal sheep liver**. Drug Metab Dispos 1996;24(12):1378-84.
CBAC COPYRIGHT: CHEM ABS An isolated, perfused fetal sheep liver model (recirculating) was used to study the metab. and disposition of p-nitrophenol (PNP). PNP was added to the reservoir at 14, 72, or 144 muM. Samples were taken from the reservoir every 5-10 min, and all bile was collected at 15-30-min intervals. Elimination of PNP from the perfusate demonstrated Michaelis-Menten kinetics, and the calcd. pharmacokinetic parameters for PNP elimination were: KM 13.0 muM, Vmax 32.1 nmol/min/g liver, and intrinsic clearance 3.39 mL/min/g. At the end of the 120-min perfusion period, PNP could be accounted for entirely as PNP sulfate (PNP-S) and PNP glucuronide (PNP-G). The perfusate ratio of PNP-S to PNP-G at 120 min was 2.21 at 14 muM PNP, 0.86 at 72 muM, and 0.31 at 144 muM, because of satn. of sulfate prodn. with increasing dose. PNP-S and PNP-G were eliminated in the bile in small amts. (<3% of the dose), and the PNP-S/PNP-G ratio in bile was 1. It is conclude that: near-term fetal sheep liver can metabolize PNP to PNP-G and PNP-S with efficiencies that may be comparable to those of adults; as in adults, fetal sulfation is of low capacity, relative to glucuronidation; unlike adults, fetuses have little capacity to transport the PNP-G formed in the hepatocytes into bile.

Thompson MB. **Bile acids in the assessment of hepatocellular function**. Toxicol Pathol 1996; 24 (1):62-71.
Bile acids, which are synthesized in the liver from cholesterol, are important in the production of bile flow, excretion of cholesterol, and intestinal digestion and absorption of fats and fat-soluble vitamins. Increases and/or alterations in concentrations of bile acids in serum are specific and sensitive indicators of hepatobiliary disorders. Synthesis of bile acids in hepatocytes involves steps in endoplasmic reticulum, cytosol, mitochondria, and peroxisomes. Other important hepatocellular processes involving bile acids include active uptake by the basolateral membrane, intracellular transport, P-450-mediated conjugations and hydroxylations, and canalicular secretion. Hydrophobic bile acids produce hepatotoxicity in vivo and in vitro. In experimental and epidemiologic studies, some of these forms have been identified as causative agents in the development of colon and liver (experimental only) cancer. Conversely, several hydrophilic forms, primarily ursodeoxycholic acid, have demonstrated cytoprotective properties in a variety of clinical and experimental hepatobiliary diseases and disorders. Because bile acids can have dramatically different properties and effects, determination of mechanisms

of action of these compounds has become an active area of research. Primary isolated hepatocytes provide an opportunity to investigate bile acid-related functions and effects in well-designed, carefully controlled studies. Short-term cultures have been used to study a variety of issues related to bile acids, including cytotoxicity, synthesis, and hepatocellular processing. With these systems, however, many functions of mature hepatocytes, including those pertaining to bile acids, can be lost when cultures are maintained for more than several days. Recent developments in culture techniques permit long-term maintenance of functionally stable, differentiated cells. Pertaining to bile acid research, these systems remain to be fully characterized but, in appropriate situations, they should provide important alternatives to in vivo studies and short-term in vitro assays.

Turesky RJ, Gremaud E, Markovic J, Snyderwine EG. **DNA adduct formation of the food-derived mutagen 2-amino-3-methylimidazo[4,5-f]quinoline in nonhuman primates undergoing carcinogen bioassay**. Chem Res Toxicol 1996;9(2):403-8.
DNA adduct formation of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) was investigated in cynomolgus monkeys. The pattern and distribution of DNA adducts examined by 32P-postlabeling were similar in all tissues 24 h after a single oral dose of IQ (20 mg/kg). The highest DNA adduct levels were found in the liver (3.67-11.19 adducts per 10(7) bases), followed by kidney (0.53-1.16 adducts per 10(7) bases), with comparable adduct levels detected in colon, heart, and pancreas (0.15-0.40 adducts per 10 (7) bases). Two 2'-deoxyguanosine (dG) adducts accounted for approximately 90% of the observed lesions in all tissues. N-(Deoxyguanosin-8-yl)-2-amino-3-methylimidazo[4,5-f]quinoline (dG-C8-IQ) was the major adduct and accounted for approximately 50-80% of the adducts, followed by 5-(deoxyguanosin-N2-yl)-amino-3-methylimidazo[4,5-f]quinoline (dG-N2-IQ) which accounted for 20-40% of the adducts. DNA adduct formation was also investigated in animals undergoing carcinogen bioassay with IQ administered at 10 or 20 mg/kg, 5 days per week for up to 9.2 years. In chronically treated animals, the DNA adduct levels in pancreas, kidney, and heart increased on average by 40- to 90-fold over those observed in animals given a single dose, while only 3- to 10-fold increases in adducts were observed in colon and liver. A sharp increase in the contribution of dG-N2-IQ to total DNA adducts occurred in all slowly dividing tissues during chronic treatment, and dG-N2-IQ became the predominant lesion. There was no preferential accumulation of dG-N2-IQ in the colon, a tissue with a high rate of cell division, and dG-C8-IQ remained the predominant lesion. These findings point to a preferential removal of the dG-C8-IQ adduct by enzyme repair system(s) in slowly dividing tissues. The respective roles of dG-N2-IQ and dG-C8-IQ, and the involvement of adduct repair in the potent hepatocarcinogenicity of IQ, merit further investigation.

Yamazoe Y, Nagata K, Ozawa S, Gong DW, Kato R. **Activation and detoxication of carcinogenic arylamines by sulfation**. Princess Takamatsu Symp 1995;23:154-62.
Hepatic sulfation of heterocyclic and non-heterocyclic arylamines was studied to assess enzymes responsible for their metabolisms. Both 2-amino-3-methylimidazo[4,5-f]quinoline (IQ)- and non-IQ-type (beta-carboline) heterocyclic amines were N-sulfated to form their sulfamates in cytosols of rat livers in the presence of 3'-phosphoadenosine-5'-phosphosulfate (PAPS). An arylsulfo-transferase, ST1A1, whose cDNA was isolated from a rat cDNA library, was expressed in COS-1 cells. The expressed enzyme catalyzed N-sulfation of IQ, but not appreciably those of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 2-amino-6-methyldipyrido[1,2-a:3',2'-d]imidazole (Glu-P-I), and

3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2). N-Sulfation of heterocyclic amines except IQ was higher in hepatic cytosols of female rats than of male rats. These results suggest the involvement of at least plural forms of sulfotransferase on the N-sulfation. In addition, N-sulfation of IQ was also observed in cytosol of a human liver, suggesting that N-sulfation is one of the metabolic pathways of heterocyclic amines in humans as well as rats. Hepatic sulfotransferase also catalyzes metabolic activation of N-hydroxy derivatives of carcinogenic arylamines. Using anti-HAST (hydroxylarylamine sulfotransferase) antibodies and ST1A1 cDNA as screening probes, several cDNA clones were isolated from the cDNA library. A new member of arylsulfotransferase, ST1C1, whose cDNA shows considerable sequence similarity to ST1A1 cDNA, was found to catalyze O-sulfation of N-hydroxy-2-acetylaminofluorence by the cDNA expression in COS-1 cells. From the close similarity of ontogenic profile and sex-specific expression of ST1C1 and HAST, ST1C1 cDNA was shown to encode a major sulfotransferase (HAST) mediating the metabolic activation of N-hydroxyarylamines in rat livers. In addition, properties of PAPS-dependent N-hydroxyarylamine activation and sulfotransferase in human livers are also discussed.

IMMUNOTOXICITY

Chandler JC, Frankel AE, Tagge EP. **Genetic engineering of immunotoxins** Semin Pediatr Surg 1996;5 (3):206-11.
Immunotoxins, composed of both targeting mechanisms and toxins, hold great promise for the treatment of some cancers and other pathological conditions. Research and manufacture of these drugs use various techniques of molecular biology, some of which are described in this article. A considerable amount of research has focused on ricin, a plant toxin, and its immunoconjugates. Areas currently under evaluation by our laboratory include structure-function studies based on mutational analysis, enhancement of intracellular trafficking, genetic fusion of the targeting and toxic moieties, and the expression of toxin in plant cell culture.

Sikorski EE, Gerberick GF, Ryan CA, Miller CM, Ridder GM. **Phenotypic analysis of lymphocyte subpopulations in lymph nodes draining the ear following exposure to contact allergens and irritants**. Fundam Appl Toxicol 1996;34(1):25-35.
BIOSIS COPYRIGHT: BIOL ABS. The murine local lymph node assay (LLNA) measures in vivo proliferation in draining lymph nodes (DLN) following topical exposure to chemicals to assess contact sensitization potential. However, proliferation has also been observed with some irritants. To further characterize events in the DLN during the LLNA and distinguish allergens from irritants, phenotypic analysis of lymphocyte subsets was made following topical exposure. In preliminary studies, mice were treated on the ears for 3 consecutive days, and 48 hr following the final application, analysis of CD3, CD4, CD8, and B220 expression was evaluated by flow cytometry. The allergens oxazolone (OXAZ) and picryl chloride (TNCB) and the irritant benzalkonium chloride (BC) increased cell number compared to vehicle. The increase in lymph node cellularity for these materials was due to an increase in the total number of T and B lymphocytes. Interestingly, even though contact sensitization is a cell-mediated immune response (Th1), mice exposed to the contact allergens showed a preferential increase in B lymphocytes in the DLN as seen by an increase in the percentage of B220+ cells. The percentage of B220+ cells was 13.1 and 36.1% for OXA and TNCB, respectively, compared to percentages of 7.4 and

9.3% for irritant and vehicle, respectively. With some allergens, a concomitant decrease in the percentage of CD3+ cells was seen. Time course studies demonstrated the increase in the percentage of B220+ cells was seen in allergen treated mice by 24 hr after the final application of material, plateaued by 48 hr, and was still elevated by 96 hr. In allergen-treated mice, percentages of B220+ cells increased dose dependently. Further studies were performed to evaluate additional contact allergens and irritants and determine if evaluation of flow cytometric parameters could potentially identify contact allergens and differentiate them from irritants. Analysis of data from these studies, which examined a total of five contact allergens and six irritants, showed that the modifications to the LLNA improved the identification of irritants and allergens in individual experiments by including both phenotypic analysis of the DLN and cell number per node as endpoints rather than either endpoint alone.

Westwood FR, Jones DV, Aldridge A. **The synovial membrane, liver, and tongue: target organs for a ricin A-chain immunotoxin (ZD0490)**. Toxicol Pathol 1996;24(4):477-83.
ZD0490 is an immunotoxin consisting of a mouse monoclonal antibody conjugated to recombinant ricin A-chain (rRAC). It was developed at Zeneca Pharmaceuticals as a treatment for certain antigen-bearing tumors. During safety evaluation studies in rats, a number of reversible inflammatory changes were seen. The synovial membranes of articular joints showed a marked degeneration and necrosis with an associated inflammation. When of mild severity only the synovial membrane was involved, but when more severe many adjacent tissues including the surface of the articular cartilage were affected. Some nonspecific skeletal muscle toxicity occurred. However, tongues from the intravenously (tail) dosed rats consistently showed inflammation specifically located in the ventral subepithelial area with myocyte degeneration and necrosis. Also, hepatic peliosis primarily located in the subcapsular areas was induced. Studies with rRAC alone indicated that ricin A-chain (RAC) is the component responsible for these findings. It is suggested that cells of a macrophage type with the ability to specifically bind RAC may at least in part determine the location and nature of the changes seen.

NEUROTOXICITY

Abdelilah S, Mountcastle-Shah E, Harvey M, Solnica-Krezel L, Schier AF, Stemple DL, Malicki J, Neuhauss SC, Zwartkruis F, Stainier DY, et al. **Mutations affecting neural survival in the zebrafish Danio rerio**. Development 1996;123:217-27.
Programmed cell death is a prominent feature of normal animal development. During neurogenesis, naturally occurring cell death is a mechanism to eliminate neurons that fail to make appropriate connections. To prevent accidental cell death, mechanisms that trigger programmed cell death, as well as the genetic components of the cell death program, are tightly controlled. In a large-scale mutagenesis screen for embryonic lethal mutations in zebrafish Danio rerio we have found 481 mutations with a neural degeneration phenotype. Here, we present 50 mutations that fall into two classes (termed spacehead and fala-like) that are characterized by two main features: first, they appear to affect cell survival primarily within the neuroectodermal lineages during somitogenesis, and second, they show an altered brain morphology at or before 28 hours of development. Evidence for the specificity of cell death within the central nervous system comes from visual inspection of dying cells and analysis of DNA fragmentation, a process associated with apoptotic cell death. In mutants, the level of dying cells is

significantly increased in brain and spinal cord. Furthermore, at the end of somitogenesis, the cell count of radial glia and trigeminal neurons is reduced in some mutants of the spacehead class. A variety of neurodegenerative disorders in mouse and humans have been associated with abnormal levels of programmed cell death within the central nervous system. The mutations presented here might provide a genetic framework to aid in the understanding of the etiology of degenerative and physiological disorders within the CNS and the activation of inappropriate programmed cell death.

Binding N, Madeja M, Musshoff U, Neidt U, Altrup U, Speckmann EJ, Witting U. **Prediction of neurotoxic potency of hazardous substances with a modular in vitro test battery**. Toxicol Lett 1996;88(1-3):115-20.
Neurotoxic action was investigated on different model nervous systems linked to a modular in vitro test battery. Voltage operated potassium channels and glutamate operated ion channels expressed in oocytes of the clawed frog Xenopus laevis by injection of cRNA (cloned RNA) or mRNA, respectively, as well as isolated neurons and isolated neuronal networks from the buccal ganglia of the snail Helix pomatia, were used as consecutive modules of different complexity. Lead (Pb2+) was chosen as a known neurotoxic model substance to evaluate the suitability of the test battery to predict the neurotoxic potency of hazardous substances, to establish dose-response relationships, and to investigate the basic mechanisms involved in neurotoxicity. All modules delivered consistent results: potassium currents were reduced by lead with a threshold concentration of 0.1 mumol/l. Membrane currents elicited by the glutamate receptor agonists kainate were decreased by lead with a threshold concentration below 0.1 mumol/l, while currents elicited by the agonist AMPA were not affected. Action potentials generated by the isolated B4 snail neuron showed a decrease of potential amplitude and a prolongation of potential duration after application of lead. The neuronal network controlling the feeding activities of the snail reacted with a decrease of the frequency of the spontaneously generated feeding depolarisations, thus showing the direct neurotoxic effect of lead on body functions and behaviour.

Bonhaus DW, Herman RC, Brown CM, Cao Z, Chang LF, Loury DN, Sze P, Zhang L, Hunter JC. **The beta 1 sodium channel subunit modifies the interactions of neurotoxins and local anesthetics with the rat brain IIA alpha sodium channel in isolated membranes but not in intact cells**. Neuropharmacology 1996;35(5):605-13.
Mammalian brain sodium channels consist of an alpha subunit and two smaller beta subunits. The role of the beta 1 subunit in modulating ligand interactions at these channels was examined using a cell line stably expressing human beta1 and rat brain IIA alpha subunits. Coexpression of the beta 1 subunit had no effect on the potencies of sodium channel blockers in inhibiting whole cell [3H]batrachotoxinin A benzoate ([3H]BTX) binding or veratridine-stimulated [14C]guanidinium influx. Coexpression of the beta 1 subunit also had no effect on the potencies of alpha scorpion toxin, brevetoxin, or RU 39568 in stimulating [14C]guanidinium influx. By contrast, coexpression of the beta 1 subunit had dramatic effects on ligand interactions in isolated membranes. In isolated membranes of cells expressing only the alpha subunit, the neurotoxins had no stimulatory effect on [3H]BTX binding and the potencies of local anesthetic-like channel inhibitors were 10-100-fold lower than those at native sodium channels. Whereas in membranes of cells coexpressing the beta 1 subunit, the neurotoxins increased [3H]BTX binding 30-fold and the potencies of the sodium channel inhibitors closely matched those found at native sodium channels. These findings indicate that the beta 1 subunit is not required for the binding of sodium

channel activators or inhibitors but rather, that the beta 1 subunit may stabilize the alpha subunit in a functional conformation thereby allowing detection of these interactions in disrupted membranes.

Brown DR, Schmidt B, Kretzschmar HA. **A neurotoxic prion protein fragment enhances proliferation of microglia but not astrocytes in culture**. Glia 1996;18(1):59-67.
The scrapie isoform of the prion protein (PrPSc) induces pathological changes in the central nervous system including neurodegeneration and gliosis. A synthetic prion protein (PrP) peptide corresponding to amino acid residues 106-126 has been shown to be toxic to neurons that express PrPC, the cellular isoform of PrP. Here we show that in mixed glial cultures PrP106-126 induces astroglial proliferation that is dependent on cellular PrPc expression. In purified cultures of glial subtypes only microglia proliferated in response to PrP106-126. This effect was independent of PrP expression. Destruction of microglia in mixed glial cultures by L-leucine methyl ester (LLME) treatment abolished enhanced proliferation caused by PrP106-126. This proliferative effect can be restored by co-culturing LLME-treated astrocytes with microglia. Microglia therefore seem to mediate the proliferative effect exerted by PrP106-126 on astrocytes.

Dorandeu F, Pernot-Marino I, Lallement G. **[Effects of the beta-neurotoxin paradoxin on neurotransmitter uptake in vitro: I. Glutamate uptake impairment]**. Trav Sci Cherch Service Sante Armees 1996;(17):125-6. (Fre)
BIOSIS COPYRIGHT: BIOL ABS. Paradoxin (PDX), isolated from the venom of the Australian Elapid Oxyuranus microlepidotus, belongs to the beta-neurotoxin family (neurotoxins with phospholipase A2, PLA2 activity). Some of these toxins exert seizure-inducing properties. In the search for neurochemical bases for such effect, we were interested in addressing the possible action of PDX on glutamate reuptake as this excitatory amino acid is very well involved in many epilepsy models. In a nominally calcium-free medium, PDX (1-200 nM) was able to significantly decrease labelled glutamate uptake by hippocampal mini-slices. This suggests a non-catalytically-mediated impairement of uptake. Furthermore, we found no evidence of competition between PDX and (3H)L-Aspartate that binds to glutamate carrier proteins. A direct blockage of uptake by binding to the carrier proteins is thus unlikely to occur.

Dorandeu F, Pernot-Marino I, Lallement G. **[Effects of the beta-neurotoxin paradoxin on neurotransmitter uptake in vitro: II. Choline and dopamine uptake impairment]**. Trav Sci Cherch Service Sante Armees 1996;(17):127-8. (Fre)
BIOSIS COPYRIGHT: BIOL ABS. Paradoxin (PDX), isolated from the venom of the Australian Elapid Oxyuranus microlepidotus, belongs to the beta-neurotoxin family. In spite of a claimed cholinergic specificity for these toxins, several works tended to show that such specificity may not exist in the central nervous system. The seizure-inducing properties of some members of this family urged us to investigate PDX effects on choline uptake in an in vitro hippocampal preparation. Furthermore, some behavioral signs observed after in vivo administration of PDX led us to study possible action of the toxin on dopamine uptake by striatal mini-slices. We show here that in a nominally calcium-free medium, PDX (1-200 nM) was able to significantly decrease labelled choline and dopamine uptake by these preparations. This suggests a non-catalytically-mediated effect on uptake. Nevertheless, a direct blockage of uptake by binding to the carrier proteins is unlikely to occur.

Ferreira IL, Duarte CB, Carvalho AP. **Ca2+ influx through glutamate receptor-associated channels**

in retina cells correlates with neuronal cell death. Eur J Pharmacol 1996;302(1-3):153-62.
We studied the effect of glutamate, N-methyl-D-aspartate (NMDA), kainate or K+ depolarization, on neurotoxicity in cultured chick retinal cells, under conditions in which we could discriminate between Ca2+ entering through ionotropic glutamate receptors and voltage-sensitive Ca2+ channels (VSCCs). When neurons were challenged with NMDA, kainate or glutamate, in Na(+)-containing medium, a decrease in cell survival was observed, whereas K+ depolarization did not affect the viability of the cells. The Mg2+ ion completely prevented the toxic effect mediated by the NMDA receptor, and had a small but significant protective effect at the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/kainate (AMPA/kainate) receptor-induced cell death. We observed that, in a Na(+)-free N-methyl-D-glucamine (NMG) medium, to avoid the activation of VSCCs indirectly by the glutamate receptor agonists, stimulation of the glutamate receptors causes Ca2+ influx only through NMDA and AMPA/kainate receptor-associated channels, and that Ca2+ entry correlates well with subsequent cell death. These results show that the activation of NMDA or AMPA/kainate receptors can cause excitotoxicity in retinal neurons by mechanisms not involving Na+ influx, but rather depending on the permeation of Ca2+ through glutamate receptor-associated channels. For small Ca2+ loads the entry of Ca2+ through the NMDA receptor-associated channel was more efficient in triggering cell death than the influx of Ca2+ through the AMPA/kainate receptor.

Grange-Messent V, Raison D, Bouchaud C. **Compared effects of extracellular K+ ions and soman, a neurotoxic, on cerebral astrocyte morphology**. An in vitro study. J Submicrosc Cytol Pathol 1996; 28 (2):151-9.
One of the roles of astrocytes in the maintenance of perineuronal ionic balance during intense neuronal activity occurring after injection of convulsant agents like soman. Soman is an irreversible cholinesterase inhibitor and induces brain damage with early swelling of astrocytic perivascular processes. Mature astrocytes are easily characterized on freeze-fracture replicas owing to the presence of regular geometric aggregates of intramembranous particles: the 'orthogonal arrays' (OAs). In primary cultures of astrocytes OA distribution is homogeneous throughout the plasma membrane. A present hypothesis (see review in Risau and Wolburg, 1990) considers that these OAs are associated with channels controlling potassium ion concentration in the cerebral parenchyma. We have investigated the effects of extracellular concentrations of K+ ions identical to those observed during neuronal activity on primary cultures of astrocytes and effects induced by soman. High concentrations of K+ ions (60 mM) as well as soman exerted direct effects on astrocytic plasma membranes: K+ ion influx within astrocytes induces a partial disaggregation of OAs and more acutely than soman. Neither K+ ions nor soman induce swelling of astrocytic end-feet.

Hiel H, Elgoyhen AB, Drescher DG, Morley BJ. **Expression of nicotinic acetylcholine receptor mRNA in the adult rat peripheral vestibular system**. Brain Res 1996;738(2):347-52.
CBAC COPYRIGHT: CHEM ABS The mRNA expression of the neuronal nicotinic acetylcholine receptor subunits was detd. in adult rat vestibular end-organs and in Scarpa's ganglion (SCG) by in situ hybridization with [35S]riboprobes. Neurons in the SCG expressed the alpha4-7 and beta2-3 mRNAs, but not alpha3 or beta4 mRNAs. Not all SCG neurons expressed every mRNA found in SCG. The alpha6 and beta2-3 riboprobes labeled all neurons, but alpha4, alpha5, and alpha7 mRNAs were selectively expressed in one or more subpopulations of SCG neurons. Vestibular sensory hair cells, in

contrast, expressed only alpha9 mRNA.

Isu Y, Nagashima U, Aoyama T, Hosoya H. **Development of neural network simulator for structure--activity correlation of molecules (NECO). Prediction of endo/exo substitution of norbornane derivatives and of carcinogenic activity of PAHs from 13C-NMR shifts**. J Chem Inf Comput Sci 1996;36(2):286-93.
A perceptron type neural network simulator for structure--activity correlation of molecules has been developed with two different learning methods, i.e., back-propagation and reconstruction methods. First by use of the back-propagation method the exo/endo branching of norbornane and norbornene derivatives was correctly predicted from the set of 13C NMR chemical shifts for various ring carbon atoms. Then the obtained correlation was analyzed by the reconstruction learning method. It was shown in this case that the NMR shifts for two carbon atoms out of seven have strong correlation with the exo/endo branching. Further, structure--activity correlation between the 13C NMR chemical shifts and carcinogenicity of 11 polycyclic aromatic hydrocarbons was also analyzed using the reconstruction method. It was demonstrated that neural network analysis is suitable for the elucidation of complicated structure--activity problems where many factors are nonlinearly entangled.

Ji YH, Mansuelle P, Terakawa S, Kopeyan C, Yanaihara N, Hsu K, Rochat H. **Two neurotoxins (BMK I and BMK II) from the venom of the scorpion Buthus martensi Karsch: purification, amino acid sequences and assessment of specific activity**. Toxicon 1996;34(9):987-1001.
BIOSIS COPYRIGHT: BIOL ABS. Two neurotoxins, BmK I and BmK II, were purified from the venom of the Chinese scorpion Buthus martensi Karsch. The complete amino acid sequences of both toxins, each containing 64 amino acid residues, were determined by the automatic sequencing of reduced and S-carboxymethylated toxins and their peptides, obtained after cleavage with TPCK-treated trypsin and Staphylococcus aureus V8 protease, respectively. Toxicity as minimum lethal dose tested by i.c.v. injection in mice showed that BmK I was six times more potent than BmK II. Only two amino acid replacements were found: at position 59 Val in BmK I was replaced by Ile in BmK II, and at position 62 a basic Lys residue in BmK I was substituted by a neutral Asti residue in BmK II. These features suggest that the positively charged residue (Lys or Arg) in the C-terminal position 62 (or 61 or 63) may also play an important role in facilitating the interaction between scorpion neurotoxins and the receptor on sodium channels. The effects of BmK I on nerve excitability were examined with the crayfish axon using intracellular recording and voltage-clamp conditions. The results indicate that BmK I preferentially blocks the sodium channel inactivation process. Thus, functional and structural similarities suggest that BmK I and BmK II belong to group 3 of scorpion alpha-type toxins.

Jiang YJ, Brand M, Heisenberg CP, Beuchle D, Furutani-Seiki M, Kelsh RN, Warga RM, Granato M, Haffter P, Hammerschmidt M, et al. **Mutations affecting neurogenesis and brain morphology in the zebrafish, Danio rerio**. Development 1996;123:205-16.
In a screen for embryonic mutants in the zebrafish a large number of mutants were isolated with abnormal brain morphology. We describe here 26 mutants in 13 complementation groups that show abnormal development of large regions of the brain. Early neurogenesis is affected in white tail (wit). During segmentation stages, homozygous wit embryos display an irregularly formed neural keel, particularly in the hindbrain. Using a variety of molecular markers, a severe increase in the number of various early differentiating neurons can be demonstrated. In contrast, late differentiating neurons, radial

glial cells and some nonneural cell types, such as the neural crest-derived melanoblasts, are much reduced. Somitogenesis appears delayed. In addition, very reduced numbers of melanophores are present posterior to the mid-trunk. The wit phenotype is reminiscent of neurogenic mutants in Drosophila, such as Notch or Delta. In mutant parachute (pac) embryos the general organization of the hindbrain is disturbed and many rounded cells accumulate loosely in the hindbrain and midbrain ventricles. Mutants in a group of 6 genes, snakehead(snk), natter (nat), otter (ott), fullbrain (ful), viper (vip) and white snake (wis) develop collapsed brain ventricles, before showing signs of general degeneration. atlantis (atl), big head (bid), wicked brain (win), scabland (sbd) and eisspalte (ele) mutants have different malformation of the brain folds. Some of them have transient phenotypes, and mutant individuals may grow up to adults.

Karlstrom RO, Trowe T, Bonhoeffer F. **Genetic analysis of axon guidance and mapping in the zebrafish**. Trends Neurosci 1997;20(1):3-8.
CBAC COPYRIGHT: CHEM ABS Systematic genetic screens have been powerful tools in identifying genes responsible for axon guidance in fruitflies and nematodes. This approach has now been extended to the study of axon guidance and the formation of topog. neuronal connections in the vertebrate brain. A systematic genetic screen was used to identify genes responsible for precise axon pathfinding and targeting in the retinotectal system of one zebrafish (Danio rerio). Over 30 genes were found that affect either: (1) retinal axon pathfinding to the contralateral tectal lobe; or (2) the topog. connection between the eye and the tectum. The zebrafish retinotectal mutants represent a new resource for the study of axon guidance in the vertebrate brain.

Kuchel GA, Rowe W, Meaney MJ, Richard C. **Neurotrophin receptor and tyrosine hydroxylase gene expression in aged sympathetic neurons**. Neurobiol Aging 1997;18(1):67-79.
CBAC COPYRIGHT: CHEM ABS RNase protection measurements revealed decreases of 26% in p75 neurotrophin receptor mRNA and 30% in trkA mRNA in superior cervical ganglia (SCG) of aged Long-Evans rats. These declines were not related to the presence of a spatial memory impairment, whose presence is known to strongly predict increased hypothalamic-pituitary-adrenal axis activity in these aged animals. A similar decrease with age was obsd. in p75, but not cyclophilin mRNA levels in SCG from F-344 inbred rats. In situ hybridization with paired sections from mature and aged F-344 rats revealed a 25% decline in the mean neuronal labeling index (LI) for p75 mRNA. In other paired sections, mean trkA LI decreased 16%, tyrosine hydroxylase (TH) LI increased 74% and cyclophilin LI did not change. Neuronal hypertrophy, p75 decreases and TH increases all occurred to a greatest extent in intermediate-sized neurons, resembling those innervating the pineal and cerebral vessels. In contrast to other SCG targets, this innervation is known to decline nearly 50% with aging. Retrograde tracer/in situ hybridization studies will be required to establish whether decreased p75 represents a marker for selective axonal regression and also to det. the significance of increased TH and neuronal hypertrophy.

Liu QS, He XP, Liu CG. **[Nicotinic currents of cultured rat superior cervical ganglion neurons and use-dependent block by mecamylamine]**. Chung Kuo Yao Li Hsueh Pao 1995;16(6):520-3 . (Chi)
AIM: Comparison of action of nicotinic agonists and antagonists on nicotinic acetylcholine receptor (nAChR) in superior cervical ganglion (SCG) neurons. METHODS: Whole-cell recordings were made from cultured neonatal rat SCG neurons. Cholinergic drugs were applied by local pressure perfusion. RESULTS: The neurons were activated by nicotinic agonists and peak current were acetylcholine (ACh), 443 +/- 183 pA; nicotine, 1175 +/- 377 pA; dimethylphenylpiperazinium (DMPP), 2946 +/- 358

pA, respectively. The nicotinic responses were blocked by mecamylamine (Mec), hexamethonium and curare, the efficacies were 435 +/- 154 pA, 725 +/- 320 pA, 887 +/- 214 pA, but not by alpha-bungarotoxin. The block by Mec was use-dependent, i.e., it was dependent on repeated presentation of the agonists. The first 6 peak currents were expressed as percentage of the first response as following: 100, 64 +/- 3, 50 +/- 3, 41 +/- 4, 32 +/- 3%. CONCLUSION: The present data suggest that nAChR of SCG neurons have different pharmacological characteristics from that of muscle or central neurons.

Matteoli M, Verderio C, Rossetto O, Iezzi N, Coco S, Schiavo G, Montecucco C. **Synaptic vesicle endocytosis mediates the entry of tetanus neurotoxin into hippocampal neurons**. Proc Natl Acad Sci USA 1996;93(23):13310-5.
BIOSIS COPYRIGHT: BIOL ABS. Tetanus neurotoxin causes the spastic paralysis of tetanus by blocking neurotransmitter release at inhibitory synapses of the spinal cord. This is due to the penetration of the toxin inside the neuronal cytosol where it cleaves specifically VAMP/synaptobrevin, an essential component of the neuroexocytosis apparatus. Here we show that tetanus neurotoxin is internalized inside the lumen of small synaptic vesicles following the process of vesicle reuptake. Vesicle acidification is essential for the toxin translocation in the cytosol, which results in the proteolytic cleavage of VAMP/ synaptobrevin and block of exocytosis.

Noah CW, Poteet SS, Ramos NC, Perez JC, Huang SY. **Production of monoclonal antibodies specific to Clostridium botulinum type B neurotoxin**. J AOAC Int 1995;78(2):381-5.
BIOSIS COPYRIGHT: BIOL ABS. Four monoclonal antibodies were produced for use in a rapid method to detect Clostridium botulinum type B neurotoxin. Cells of mouse myeloma cell line SP2/0 were fused with splenocytes of immunized BALB/c mice. An immunoblot assay of semipurified commercial neurotoxins of C. botulinum types A, B, C, D, E, and F was used to show specificity. All the monoclonal antibodies reacted with type B neurotoxin but did not cross-react with the other types. The monoclonal antibodies, separately and combined, did not neutralize the toxin in mice, and all showed specificity to the whole neurotoxin molecule and the heavy-chain component by immunoblot. No evidence of specific binding to the hemagglutinin molecule was noted. When tested against concentrated cultured supernatants of C. botulinum types A, B, E, and F, the 4 monoclonal antibodies reacted only against type B strains. They will be incorporated into a rapid assay with other specific monoclonal antibodies to detect C. botulinum neurotoxins from pure cultures or suspect foods.

Okuda S, Nishiyama N, Saito H, Katsuki H. **Hydrogen peroxide-mediated neuronal cell death induced by an endogenous neurotoxin, 3-hydroxykynurenine**. Proc Natl Acad Sci USA 1996;93 (22):12553-8.
3-Hydroxykynurenine (3-HK) is a tryptophan metabolite whose level in the brain is markedly elevated under several pathological conditions, including Huntington disease and human immunodeficiency virus infection. Here we demonstrate that micromolar concentrations (1-100 microM) of 3-HK cause cell death in primary neuronal cultures prepared from rat striatum. The neurotoxicity of 3-HK was blocked by catalase and desferrioxamine but not by superoxide dismutase, indicating that the generation of hydrogen peroxide and hydroxyl radical is involved in the toxicity. Measurement of peroxide levels revealed that 3-HK caused intracellular accumulation of peroxide, which was largely attenuated by application of catalase. The peroxide accumulation and cell death caused by 1-10 microM 3-HK were also blocked by pretreatment with allopurinol or oxypurinol, suggesting that endogenous xanthine

oxidase activity is involved in exacerbation of 3-HK neurotoxicity. Furthermore, NADPH diaphorase-containing neurons were spared from toxicity of these concentrations of 3-HK, a finding reminiscent of the pathological characteristics of several neurodegenerative disorders such as Huntington disease. These results suggest that 3-HK at pathologically relevant concentrations renders neuronal cells subject to oxidative stress leading to cell death, and therefore that this endogenous compound should be regarded as an important factor in pathogenesis of neurodegenerative disorders.

Shapiro MS, Zhou J, Hille B. **Selective disruption by protein kinases of G-protein-mediated Ca2+ channel modulation**. J Neurophysiol 1996;76(1):311-20.
1. We studied the effects of phorbol-12-myristate, 13-acetate (PMA) on G-protein-mediated inhibition of Ca2+ channels by several neurotransmitters in rat superior cervical ganglion (SCG) sympathetic neurons, with the use of the whole cell patch clamp. PMA attenuated membrane-delimited inhibition of calcium currents (ICa) by norepinephrine (NE) and somatostatin by more than half, but did not attenuate inhibition by M1 muscarinic receptors, which use a diffusible cytoplasmic messenger. Inhibition of ICa by NE through pertussis-toxin-sensitive and -insensitive G proteins was equally attenuated by PMA. PMA enhanced ICa in about half the neurons (enhancement of 10 +/- 1%, mean +/- SE) and strongly reduced the holding current in 44 of 61 cells. 2. The M-type K+ current (IM) was not suppressed by PMA, and PMA did not attenuate inhibition of IM by muscarinic agonists, which is also via a diffusible cytoplasmic messenger. 3. Attenuation of NE and somatostatin inhibition by PMA was blocked by 1 microM staurosporine, a broad-spectrum protein kinase inhibitor. Tests with three inhibitors selective for distinct isoforms of protein kinase C (PKC) gave mixed results. PMA's actions were unaffected by 1 microM calphostin C, blocked by 500 nM bisindolylmaleimide, and unaffected by the pseudosubstrate inhibitor PKC19-36. 4. Thus we find that two membrane-delimited signaling pathways that inhibit ion channels in rat SCG neurons are strongly attenuated by PMA, but signaling pathway(s) that use a diffusible cytoplasmic messenger are not. We speculate that a nonstandard PKC isoform, perhaps PKC mu, mediates PMA actions.

Shichor I, Fainzilber M, Pelhate M, Malecot CO, Zlotkin E, Gordon D. **Interactions of delta-conotoxins with alkaloid neurotoxins reveal differences between the silent and effective binding sites on voltage-sensitive sodium channels**. J Neurochem 1996;67(6):2451-60.
The delta-conotoxin-TxVIA from Conus textile (delta TxVIA) is a mollusk-specific conotoxin that slows sodium channel inactivation exclusively in mollusk neuronal membranes but reveals high-affinity binding to both mollusk (effective binding) and rat brain (silent binding) neuronal membranes, despite not having any toxic effect in vertebrates in vivo and in vitro. Using binding studies with radioactive delta TxVIA we demonstrate that a different mollusk-specific conotoxin, delta-conotoxin-GmVIA from the venom of Conus gloriamaris, possesses silent and effective binding properties in rat brain and mollusk sodium channels, respectively. Binding studies and electrophysiological tests with both vertebrate muscle and insect neuronal preparations have indicated that the silent binding sites of delta TxVIA are highly conserved in a wide range of distinct vertebrate and insect sodium channels. Direct probing of receptor site 2 by a tritiated derivative of batrachotoxin ([3H]BTX-B) revealed that [3H]BTX-B binding in mollusk sodium channels is of high affinity with no addition of enhancing ligands, unlike [3H]BTX-B binding in rat brain. In contrast to the negative allosteric modulation of delta TxVIA binding by veratridine, delta TxVIA is not able to affect the binding of [3H]BTX-B in mollusk neuronal

membranes but reduces [3H]BTX-B binding in rat brain in the presence of alpha-scorpion toxins. The latter finding indicates the existence of a pharmacological distinction between the silent and effective binding sites of delta TxVIA and points out possible functionally important structural differences between molluscan and rat brain sodium channels.

Simpson LL. **Neuropharmacological characterization of Botulinum neurotoxin**. Philadelphia: Jefferson Medical College; Contract No: DAMD17-95-C-5004. 54 P. Available From NTIS, Springfield, VA; AD-A312 072-2.
TD3: Experiments have been done to clarify the nature of botulinum toxin binding. This work is an essential prelude to efforts to identify drugs that can antagonize toxin action by blocking toxin association with cell surface receptors. During the first part of the work, experiments were done: (1) to identify human tissue preparations that can be used to study electrophysiology, ligand binding, and certain aspects of molecular biology, and (2) to examine botulinum toxin action on these tissues. This work resulted in the findings that the human pyramidalis muscle can be used as a model to study neuromuscular.

Spaniol P, Bornmann C, Hauptmann G, Gerster T. **Class III POU genes of zebrafish are predominantly expressed in the central nervous system**. Nucleic Acids Res 1996;24(24):4874-81.
CBAC COPYRIGHT: CHEM ABS POU genes encode a family of transcription factors involved in a wide variety of cell fate decisions and in the regulation of differentiation pathways. We have searched for POU genes in the zebrafish, a popular model organism for the study of early development of vertebrates. Besides five putative pseudogenes we have identified five POU genes that are expressed during embryogenesis. Probes obtained by PCR were used to isolate full-length cDNAs. Four of the isolated genes encode proteins with class III POU domains. Anal. of genomic clones suggest that the fish genes in general do not contain introns, similar to class III genes of mammals. However, the C-termini of two of the encoded proteins vary due to facultative splicing of a short intervening sequence. These two genes show very strong similarities in their sequence. They have probably arisen by gene duplication, possibly as part of a larger scale duplication of part of the zebrafish genome. Anal. of the expression of the class III genes shows that they are predominantly expressed in the central nervous system and that they may play important roles in patterning the embryonic brain.

Stanness KA, Guatteo E, Janigro D. **A dynamic model of the blood-brain barrier in vitro**. Neurotoxicology 1996;17(2):481-96.
Cell culture models have been widely used for screening of neurotoxicants and represent a viable alternative to direct in vivo experiments. We have developed a dynamic in vitro blood-brain barrier model designed to allow for extensive toxicological, pharmacological and physiological testing. Induction of blood-brain barrier properties in a tri-dimensional hollow fiber culturing apparatus was investigated by co-culturing a bovine aortic endothelial cell line (or rat brain endothelial cells) with rat brain astrocytes (or C6 rat glioma cells) under pulsatile flow conditions to mimic intraluminal blood flow. Cell growth was monitored over time by measuring glucose consumption and lactate production: these experiments confirmed that the hollow fiber cell culturing systems can maintain viable cells in culture for extended (> 1 month) periods of time. Cells were visually inspected after culturing and dissociation from the hollow fiber cartridge and identified as endothelial (by fluorescent Dil-Ac-LDL uptake) or glial (by GFAP immunoreactivity). Blood-brain barrier properties were tested by intraluminal

injection of horse-radish peroxidase (HRP, mol. weight 44,000), glucose (m.w. 180) or potassium. Either procedure demonstrated that aortic cells co-cultured with astrocytes (or C6 cells) developed a selective barrier with an estimated electrical resistance of 2,900 omega/cm2. The electrophysiological and morphological properties of BAEC were also affected by the co-culturing process, suggesting that astrocytes induced CNS properties in these cells. These results demonstrate that the hollow fiber cell co-culturing system may be used as a dynamic model of the mammalian blood-brain barrier.

Stein S, Niss K, Kessel M. **Differential activation of the clustered homeobox genes CNOT2 and CNOT1 during notogenesis in the chick**. Dev Biol 1996;180(2):519-33.
CBAC COPYRIGHT: CHEM ABS CNOT2, a newly identified homeobox gene, is phys. linked to the CNOT1 gene in the chicken genome. The two chicken genes represent two different subgroups of the Not gene family, the first including CNOT1 and the Xenopus genes XNot1 and XNot2, and the second CNOT2 and the zebrafish floating head gene. The overall expression pattern of CNOT2 in Hensen's node, notochord, neural plate, tailbud, and epiphysis resembled the CNOT1 pattern. However, several significant differences occurred: CNOT2 expression was much stronger and more widespread in the pregastrulation embryo, it showed an addnl., transient domain on the anterior intestinal portal, and lacked expression on the early anterior neural folds and the anterodistal limb bud. We studied CNOT expression by transplanting parts of the primitive streak into growing embryos or by explanting them into tissue culture. CNOT gene expression from young nodes was maintained in vivo, but required in vitro the addn. of retinoic acid. The generation of differentiated notochord structures could only be obtained, if either older node grafts were used in vitro or young node grafts were transplanted close to the primary axis in vivo. We conclude that CNOT expression in the anterior streak is not enough for notochord differentiation, but further influences are necessary. A Not-related gene has previously been isolated from Drosophila melanogaster and its expression was detected in the posterior brain and the neuroblasts (Dessian and McGinnis, 1993. Adv. Dev. Biochem. 2, 1-55). The correspondence between Not gene-expressing cells in the nervous system of Drosophila and the early neuroectoderm in the chick and its implication for a phylogenetic relationship between neuroectoderm and the notochord is discussed.

Villani L, Guarnieri T, Facchinetti F, Virgili M, Poli A. **Neurotoxic effects of DSP-4 on the noradrenergic system of the goldfish brain**. Brain Behav Evol 1996;47(5):219-24.
The substance N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) is a neurotoxin with selective and long-lasting effects on the noradrenergic (NA) neurons of mammalian brains. The present study examines the effects of this toxin on the noradrenergic system of the goldfish brain. Single doses (50 mg/kg body weight) of DSP-4 reduce the immunoreactivity of the NA synthesizing enzyme dopamine-beta-hydroxylase (DBH), as revealed by immunohistochemistry 7 and 12 days after toxin administration. The depletion involves the DBH-positive fibres and spares the DBH-positive cell bodies. Dopamine-beta-hydroxylase immunoreactivity, 40 days after toxin administration, showed a complete recovery. Ultrastructural investigations confirmed that DSP-4 toxicity affects only nervous fibres and terminals, sparing cell bodies. Administration of DSP-4 also produced a marked decrease of noradrenaline (NA) levels in the goldfish brain, seven days later, while dopamine (DA) and serotonin (5-HT) levels were unaffected by toxin injection. The reduction of NA levels induced by DSP-4 was prevented by the concomitant administration of the NA uptake inhibitor desipramine. Noradrenaline levels measured 40

days after toxin administration show that DSP-4 toxicity was completely reversed. The results suggest a pronounced plasticity of the noradrenergic system in the goldfish brain.

Yee S, Choi BH. **Oxidative stress in neurotoxic effects of methylmercury poisoning**. Neurotoxicology 1996;17(1):17-26.
Purified cultures of oligodendrocytes, astrocytes, and cerebral cortical and cerebellar granular neurons obtained from embryonic and neonatal rat brains were used to examine the effects of methylmercury-chloride (115093) (MMC) on the rate of oxygen uptake. Once exposed to MMC, the oxygen uptake in all cell types was significantly inhibited. The rate at which oxygen consumption was inhibited varied among different cell types considered, in proportion to the rate at which oxygen had been consumed by each cell type prior to exposure. The faster the normal respiratory rate, the sooner the oxygen uptake was stopped following MMC exposure. Complex specific electron donating substrates were used to stimulate the mitochondria taken from control and MMC injected rat brains in an effort to determine the effects of MMC on mitochondrial electron transport chain activity. Stimulation with complex III (ubiquinol/ cytochrome-c-oxidoreductase) caused significant increases in reactive oxygen species and thiobarbituric acid reactive substances along with a reduction in glutathione levels of MMC injected animals. Neither complex I (NADH/ubiquinone-oxidoreductase) or II (succinate/ubiquinone-oxidoreductase) produced these effects, causing the authors to suggest that MMC induces changes in electron transport in the complex III region. Cytochrome-c-reductase was inhibited in a competitive fashion. When cytochrome-c-reductase was preincubated with MMC there was no significant change in the kinetics of cytochrome-c-reductase assay. The authors suggest that mitochondria may be the first target of the neurotoxic effects of methyl-mercury and that the most likely site where excess reactive oxygen species are generated in the brain to cause oxidative stress in methyl-mercury poisoning is the mitochondrial electron transport chain.

Zedda M, Acone F, Panu R, Farina V, Sanna L, Palmieri G. **Neurotoxic effects of 3-acetylpyridine (3-AP) in the hamster (Mesocricetus auratus)**. Ital J Anat Embryol 1996;101(1):57-66.
The 3-Acetylpyridine (3-AP) is a neurotoxic that determines a selective destruction of inferior olivary nucleus in the rat. However, in the other animals (mouse, rabbit, guinea-pig, quail and chicken) the effect could be less selective in the mentioned nucleus and constant in other areas of the central and peripheral nervous system. This investigation means to expand the study of these neurotoxic effects to the hamster, species not yet studied. The intraperitoneal administration of 3-AP, at a dosage of 65 mg/ Kg, determines in this animal stridulous breathing, coughing and ataxy. Wide sites of the nervous system show, by microscopical observations, pictures of shrinking cells, vacuolization of the cytoplasm, reduction or loss of Nissl stain affinity and neuronal mutilation. The neurotoxic effects are mostly evident in the motor nuclei of the cerebral trunk and in the ventral horns of the spinal cord. However, the inferior olivary nucleus appears undamaged or sparely involved.

OCULAR TOXICITY

Lovell DP. **Principal component analysis of Draize eye irritation tissue scores from 72 samples of 55 chemicals in the ECETOC data bank**. Toxicol In Vitro 1996;10(5):609-18.

BIOSIS COPYRIGHT: BIOL ABS. The multivariate statistical method Principal Component Analysis (PCA) has been applied to a set of data from the ECETOC reference chemical data bank. PCA is a multivariate method that can be used to explore a complex data set. The results of the analysis show that most of the variability in the values for tissue damage scores for the 55 chemicals can be described by a single principal component which explains nearly 80% of the variability. This component is derived by giving approximately equal weight to each of the 18 individual measures made on the tissues over the 24-, 48- and 72-hr observation period. The principal component scores on the first component (PC I) are very highly correlated with the maximum individual weighted Draize scores or total Draize scores (TDS) derived using the Draize scoring method. A second principal component, describing about 7% of the variability, contrasts damage measured on the iris and cornea with that measured on the conjunctiva. Plots of principal component scores show the overall pattern of responses. In general, low measures of the TDS and a positive (PC I) score are associated with iris and conjunctival damage (damage to the iris was never recorded in the absence of damage to the conjunctival. High TDS and negative PC I scores are associated with corneal and/or iris and conjunctiva damage. Plots of the principal component scores identify some chemicals that appear to cause unusual patterns of damage and identify some individual animals as having outlying or idiosyncratic responses. However, the analysis suggests that (i) there is only limited evidence for differential responses of different tissues and (ii) that attempts to identify alternative tests which predict specific types of tissue damage based on the results collected in a Draize test are likely to be unsuccessful. It indicates that further refinement of the results of the in vivo Draize test will not arise from more detailed analysis of the tissue scores but by refinement in the understanding of the mechanisms associated with the test. PCA was shown to be a powerful statistical tool for the investigation of complex data sets and provides a succinct description of such data sets, allowing patterns to be identified and the potential to develop further hypotheses for investigation.

Malicki J, Neuhauss SC, Schier AF, Solnica-Krezel L, Stemple DL, Stainier DY, Abdelilah S, Zwartkruis F, Rangini Z, Driever W. **Mutations affecting development of the zebrafish retina**. Development 1996; 123:263-73.
In a large scale screen for genetic defects in zebrafish embryogenesis we identified 49 mutations affecting development of the retina. Based on analysis of living embryos as well as histological sections, we grouped the isolated mutations into six phenotypic categories. (1) Mutations in three loci result in a loss of wild-type laminar pattern of the neural retina. (2) Defects in four loci lead to an abnormal specification of the eye anlagen. Only one eye frequently forms in this class of mutants. (3) Seven loci predominantly affect development of the outer retinal layers. Mutants in this category display cell loss mainly in the photoreceptor cell layer. (4) Nine mutations cause retardation of eye growth without any other obvious abnormalities in the retina. (5) A group of twelve mutations is characterized by nonspecific retinal degeneration. (6) Four mutations display retinal degeneration associated with a pigmentation defect. Finally, two mutations, one with absence of the ventral retina and one with an eye-specific pigmentation defect, are not classified in any of the above groups. The identified mutations affect numerous aspects of eye development, including: specification of the eye anlage, growth rate of the optic cup, establishment of retinal stratification, specification or differentiation of retinal neurons and formation of the dorsoventral axis in the developing eye.

PHARMACOKINETIC AND MECHANISTIC STUDIES

Aasmundstad TA, Lillekjendlie B, Morland J. **Ethanol interference with morphine metabolism in isolated guinea pig hepatocytes**. Pharmacol Toxicol 1996;79(3):114-9.
It has previously been shown that guinea pig hepatocytes metabolise morphine in a fashion similar to humans. The metabolism of morphine (5 muM) and the formation of metabolites morphine-3-glucuronide, morphine-6-glucuronide and normorphine was studied in the absence and presence of ethanol (5, 10, 25, 60 and 100 mM) in freshly isolated guinea pig hepatocytes. In order to gain more detailed information, a mathematical model was estimated on experimental data and used to analyse the effects of ethanol on the reaction rates of the different morphine metabolites. Ethanol inhibited the rate of morphine elimination in a dose-related manner, at the high ethanol concentrations the elimination rate was 40 per cent of the control rate. The formation of morphine-glucuronides was influenced in a biphasic manner. Five and 10 mM ethanol increased both the morphine-3-glucuronide and morphine-6-glucuronide levels after 60 min incubation compared to the control, whereas at the higher ethanol concentrations (25-100 mM) the levels of morphine-glucuronides were reduced. Data from the mathematical model, however, demonstrated that the reaction rates for morphine-glucuronide formation were decreased at all ethanol concentrations and in a dose-dependent manner, the interpretation of this being that at the lower (5 and 10 mM) ethanol concentrations employed in this study, other metabolic pathways of morphine are more heavily inhibited than the glucuronidations, resulting in a shunting towards morphine-3-glucuronide and morphine-6-glucuronide. The pharmacodynamic consequences of these pharmacokinetic effects are thus somewhat difficult to predict since morphine-6-glucuronide has a higher agonist potency than morphine. At high concentrations ethanol inhibition of morphine metabolism will increase the concentration of morphine and subsequently the euphoric and the toxic effects. The lower quantities of morphine-6-glucuronide formed in the presence of high ethanol concentrations on the other hand most probably imply reduction of such effects and the net pharmacodynamic effect would be uncertain. At low ethanol concentrations, however, morphine-6-glucuronide concentrations increased and morphine metabolism was less inhibited leading to a possible potentiation of the effects of morphine. Thus, a low ethanol concentration might exert a more pronounced ethanol-drug effect interaction than a higher ethanol concentration.

Adams PC, Rickert DE. **The absorption and first-pass metabolism of (14C)-1, 3-dinitrobenzene in the isolated vascularly perfused rat small intestine**. Biopharm Drug Dispos 1996;17(8):675-98.
BIOSIS COPYRIGHT: BIOL ABS. We tested the hypothesis that the small intestine is capable of the first-pass, reductive metabolism of xenobiotics. A simplified version of the isolated vascularly perfused rat small intestine was developed to test this hypothesis with 1, 3-dinitrobenzene (1, 3-DNB) as a model xenobiotic. Both 3-nitroaniline (3-NA) and 3-nitroacetanilide (3-NAA) were formed and absorbed following intralumenal doses of 1, 3-DNB (1.8 or 4.2 mumol) to isolated vascularly perfused rat small intestine. Dose, fasting, or antibiotic pretreatment had no effect on the absorption and metabolism of 1, 3-DNB in this model system. The failure of antibiotic pretreatment to alter the metabolism of 1, 3-DNB indicated that 1, 3-DNB metabolism was mammalian rather than microfloral in origin. All data from experiments initiated with lumenal 1,3-DNB were fit to a pharmacokinetic model (model A). ANOVA analysis revealed that dose, fasting, or antibiotic pretreatment had no statistically significant effect on the model-dependent parameters. 3-NA (1.5 mumol) was administered to the lumen of isolated

vascularly perfused rat small intestine to evaluate model A predictions for the absorption and metabolism of this metabolite All data from experiments initiated with 3-NA were fit to a pharmacokinetic model (model B). Comparison of corresponding model-dependent pharmacokinetic parameters (i.e. those parameters which describe the same processes in models A and B) revealed quantitative differences. Evidence for significant quantitative differences in the pharmacokinetics or metabolism of formed versus preformed 3-NA in rat small intestine may require better definition of the rate constants used to describe tissue and lumenal processes or identification and incorporation of the remaining unidentified metabolites into the models.

Alkadhi KA, Salgado-Commissariat D, Hogan YH, Akpaudo SB. **Induction and maintenance of ganglionic long-term potentiation require activation of 5-hydroxytryptamine (5-HT3) receptors**. J Physiol (Lond) 1996;496(2):479-89.
CBAC COPYRIGHT: CHEM ABS An extracellular recording technique was used to study the effects of 5-hydroxytryptamine (5-HT) on the tetanus-induced long-term potentiation (LTP) of the nicotinic pathway of transmission in the superior cervical ganglion (SCG) of the rat. The postganglionic compd. action potential (CAP), made submaximal by treatment with hexamethonium (0.4 mM), was used as an index of transmission in the ganglion. 5-HT (10 muM) markedly enhanced the magnitude of LTP without affecting the post-tetanic potentiation (PTP). The 5-HT (2-30 muM) concn.-response curve for LTP was bell shaped as no enhancement was seen with 30 muM 5-HT. This may largely be due to activation of a 5-HT1 receptor subtype and not to desensitization. When superfused before tetanus, the 5-HT1A receptor agonist 8-hydroxydipropylaminotetralin (8-OH-DPAT, 5 muM) prevented the expression of LTP without affecting PTP. Pretreatment of ganglia with the 5-HT2 receptor agonist R-(+)-dimethoxy-4-iodoamphetamine (R-(+)-DOI, 1 muM) enhanced the tetanus-induced LTP. Similar treatment with the 5-HT2 receptor antagonist ketanserin (3 muM) had no significant effect on LTP. Pretreatment of ganglia with the 5-HT3 receptor agonist 1-m-(chlorophenyl)biguanide (m-CPBG, 1 muM), markedly increased (300%) the tetanus-induced LTP. Similar pretreatment with the 5-HT3 receptor antagonist MDL 72222 (0.5 muM) completely prevented the expression of LTP. Fully expressed LTP was reversibly blocked by MDL 72222 when applied during the maintenance phase of LTP. Tetanic stimulation of monoamine-depleted ganglia (from reserpine-pretreated rats, 3 mg kg-1 for 24 h) failed to induce LTP. In monoamine-depleted ganglia, tetanus preceded by superfusion with m-CPBG readily induced LTP. MDL 72222 completely blocked this LTP. However, in these ganglia tetanus failed to induce LTP when m-CPBG was given 2 min (during PTP) or 1 h after tetanus. Tetanic stimulation of monoamine-depleted ganglia in the presence of R-(+)-DOI failed to induce LTP. We conclude that tetanus-induced LTP of the SCG of the rat requires activation of 5-HT3 receptors both for induction and maintenance.

Alexander J, Fossum BH, Reistad R, Holme JA. **Metabolism of the food carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in the rat and other rodents**. Princess Takamatsu Symp 1995;23:113-22.
2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) is the most abundant compound of the amino-imidazoazaarens (AIA) group of muta-/carcinogens isolated from the crust of fried meat. PhIP is principally activated via P450IA2 dependent N2-hydroxylation. A major metabolic pathway is N2-glucuronidation of the proximate 2-hydroxyamino-PhIP metabolite and excretion via bile to the

intestine. After bacterial hydrolysis the proximate metabolite may be esterified by the intestinal cells and cause genetic damage. 2-Hydroxyamino-PhIP formed in vivo may be further oxidized presumably to 2-nitro-PhIP which reacts directly with glutathione through substitution of the nitro group. Detoxification is principally via P450IA1 dependent ring-hydroxylation followed by sulfation or glucuronidation. Direct glucuronidation also occurs. PhIP metabolism was examined in freshly isolated hepatocytes from rat, mouse, hamster and guinea pig. Activation was evaluated by the total level of covalent binding of PhIP to macromolecules. Rat hepatocytes had the lowest rate of metabolism, both to reactive and detoxified metabolites. The major products were 4'PhIP-sulfate, PhIP-glucuronide and 2-hydroxyamino-PhIP glucuronide, whereas in the mouse hepatocytes mainly 4'PhIP-sulfate was found. The level of covalent binding in the mouse hepatocytes exceeded those of the rat. An extensive metabolism was seen in guinea pig hepatocytes, the major products being 4'PhIP-sulfate, 4'-O-PhIP glucuronide, PhIP-glucuronide and 2-hydroxyamino-PhIP-glucuronide. The relative amount of PhIP covalently bound to macromolecules in guinea pig hepatocytes was low. Hamster hepatocytes had the highest level of covalently bound PhIP. The main metabolites were 4'PhIP-sulfate, 4'O-PhIP-glucuronide and PhIP-glucuronide. Minor amounts of 2-hydroxyamino-PhIP-glucuronide was produced in the hamster. Several unknown PhIP metabolites were formed in the hamster and guinea pig. Direct detoxification of PhIP and further metabolism of 2-hydroxyamino-PhIP to reactive and/or detoxified metabolites. The major products were 4'PhIP-sulfate, PhIP-glucuronide and 2-hydroxyamino-PhIP glucuronide, whereas in the mouse hepatocytes mainly 4'PhIP-sulfate was found. The level of covalent binding in the mouse hepatocytes exceeded those of the rat. An extensive metabolism was seen in guinea pig hepatocytes, the major products being 4'PhIP-sulfate, 4'-O-PhIP glucuronide, PhIP-glucuronide and 2-hydroxyamino-PhIP-glucuronide. The relative amount of PhIP covalently bound to macromolecules in guinea pig hepatocytes was low. Hamster hepatocytes had the highest level of covalently bound PhIP. The main metabolites were 4'PhIP-sulfate, 4'O-PhIP-glucuronide and PhIP-glucuronide. Minor amounts of 2-hydroxyamino-PhIP-glucuronide was produced in the hamster. Several unknown PhIP metabolites were formed in the hamster and guinea pig. Direct detoxification of PhIP and further metabolism of 2-hydroxyamino-PhIP to reactive and/or detoxified metabolites are important for the resulting covalent binding.

Amato G, Longo V, Mazzaccaro A, Gervasi PG. **Microsomal oxidation of N,N-diethylformamide and its effect on P450-dependent monooxygenases in rat liver**. Chem Res Toxicol 1996;9(5):882-90. N,N-Diethylformamide (DEF) is a hepatotoxic polar solvent in which metabolism has not been investigated. In this study we examined the following: (a) the oxidative metabolism of DEF using both liver microsomes from rats pretreated with selected P450 inducers and purified P450 enzyme (2B1, 2E1, 2C11); and (b) the effect of administration of DEF and its metabolite, the monoethylformamide (MEF), on induction and/or inhibition of the P450 isoforms in rats. DEF was deethylated by microsomal P450-dependent oxidation forming acetaldehyde and MEF according to Michaelis-Menten kinetic parameters. Microsomes from rats pretreated with acetone and pyrazole (selective P4502E1 inducers) or rats pretreated with dexamethasone and 200 mg/kg DEF were able to deethylate DEF in a biphasic manner, showing a low Km component with a Vmax of about 0.2 nmol/(min.mg of protein) and a Km between 70 microM and 250 microM. The low Km component was not present in control microsomes or in microsomes from rats treated with phenobarbital, beta-naphthoflavone, or clofibrate, where linear Kinetics were observed. The use of purified P4502E1 and 2C11 in a reconstituted system showed that

2E1, which oxidized DEF with a Vmax of 4.5 nmol/(min.nmol of P450) and a Km of 0.7 mM, can partially account for the low Km DEF deethylase, whereas 2C11, which oxidized DEF with a Vmax of 4.8 nmol/(min.nmol of P450) and a Km of 17 mM, might be the high Km deethylase. The purified 2B1 was barely able to deethylate DEF. A confirmation of the role of 2E1 in DEF metabolism was obtained by using various selective inhibitors of P450 isoforms and immunoprecipitation experiments with anti P4502E1 IgG. The low Km component of DEF deethylation in acetoneor pyrazole-induced microsomes was strongly inhibited (approximately 90%) by diethyldithiocarbamate, 4-methylpyrazole, and anti-2E1 IgG, but in 200 mg/kg DEF-induced microsomes the inhibition was partial, suggesting that other P450 (s) may be involved. Administration of DEF 200 mg/kg ip for 4 days induced hepatic microsomal P4502E1-dependent aniline hydroxylase, P4502B1/2-linked pentoxyresorufin O-depentylase, 16 beta-testosterone hydroxylase P4503A1/2-associated erythromycin N-demethylase, and 6 beta-testosterone hydroxylase. Alternatively, the same dose regimen of MEF induced only the aniline hydroxylase and depressed the 3A1/2-linked activities. Immunoblot experiments verified these data. These findings indicate that DEF, at low concentrations, is predominantly oxidized by P4502E1 and that this enzyme may be induced in rodents by repeated MEF or DEF treatment, thereby increasing their own metabolism and potentially their cytotoxicity through the formation of ethyl isocyanate.

Amisaki T, Eguchi S. **Pharmacokinetic parameter estimations by minimum relative entropy method**. J Pharmacokinet Biopharm 1995 Oct;23:479-94.
IPA COPYRIGHT: ASHP The minimum relative entropy method was introduced for estimating pharmacokinetic parameters, and its performance was compared with ordinary least squares and extended least squares methods using numerical simulations. Ordinary least squares was the best method and minimum relative entropy was not good when the actual observation error magnitude conformed to the assumption of ordinary least squares, that is, error variance was constant; ordinary least squares always behaved poorly with the other variance models. Minimum relative entropy performed better than least squares methods when variance of observation was proportional to its mean. Extended least squares was superior to minimum relative entropy and ordinary least squares when standard.

Andrews AM, Ladenheim B, Epstein CJ, Cadet JL, Murphy DL. **Transgenic mice with high levels of superoxide dismutase activity are protected from the neurotoxic effects of 2'-NH2-MPTP on serotonergic and noradrenergic nerve terminals**. Mol Pharmacol 1996;50(6):1511-9.
Administration of the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) analog 1-methyl-4-(2'-aminophenyl)-1,2,3,6-tetrahydropyridine (2'-NH2-MPTP; 4 x 15 mg/kg) to CD-1 mice was found to cause substantial decreases in cortical and hippocampal 5-hydroxytryptamine (5-HT) and norepinephrine (NE) to 20-30% of control 3 weeks after treatment. The magnitude of these depletions was similar to those reported previously in Swiss Webster and C57BL/6 mice given 4 x 20 mg/kg 2'-NH2-MPTP, and in keeping with these prior studies, striatal dopamine levels were unchanged by 2'-NH2-MPTP treatment in CD-1 mice. Subsequently, transgenic CD-1 mice producing high levels of human cytosolic Cu-Zn superoxide dismutase (SOD) were studied to assess the role of oxygen radicals in the mechanism of action of 2'-NH2-MPTP. In contrast to the results described above, 5-HT and NE levels were almost completely unaffected by 2'-NH2-MPTP treatment in homozygous SOD mice bearing 5-fold increases in brain SOD activity. In 2'-NH2-MPTP-treated heterozygous SOD mice, which showed an average 3-fold increase in brain SOD activity, only moderate depletions in cortical and

hippocampal 5-HT (50-60% of control) and NE (30-40% of control) were observed. Additionally, the density of [125I]RTI-55-labeled 5-HT uptake sites was studied to further assess possible 5-HT terminal loss. In various cortical and hippocampal subregions of nontransgenic mice, 5-HT uptake sites were reduced to 20-35% of control after 2'-NH2-MPTP treatment, in comparison with homozygous SOD mice, which were affected only minimally by 2'- NH2-MPTP administration, and heterozygous SOD mice, which showed intermediate reductions in 5-HT uptake site density on the order of 55-80% of control. Together, these data indicate that mice genetically endowed with increased SOD activity are protected from 2'-NH2-MPTP-induced.

Bouvier D'yvoire MJ, Maire PH. **Dosage regimens of antibacterials: implications of a pharmacokinetic/pharmacodynamic model**. Clin Drug Invest 1996;11(4):229-39.
IPA COPYRIGHT: ASHP A model that predicts dosing regimens for antibacterials using bacterial growth or kill from in vitro curve data and pharmacodynamic and pharmacokinetic models is described.

Carceles CM, Espuny A, Vicente MS, Diaz MS, Escudero E. **Single-dose pharmacokinetics of ampicillin/sulbactam (2:1) combination after intravenous administration to sheep and goats**. Res Vet Sci 1996;61(2):143-6.
CBAC COPYRIGHT: CHEM ABS The pharmacokinetic behavior of a combination of ampicillin and sulbactam (2:1) in six sheep and six goats after single i.v. doses of 20 mg kg bodyweight-1 (13.sum.33 mg kg-1 of ampicillin and 6.sum.67 mg kg-1 of sulbactam) was investigated by using a HPLC method for detg. plasma concns. The objective was to det. whether there are differences between sheep and goats in the disposition kinetics of ampicillin and sulbactam. The plasma concn.-time curves were analyzed by compartmental pharmacokinetic and noncompartmental methods. The disposition curves for both drugs were best described by a biexponential equation (2-compartment open model) in both sheep and goats. The mean (SD) elimination half-lives of ampicillin were 0.32 h in sheep and 0.32 h in goats, and the half-lives of sulbactam were 0.74 and 0.79 h in sheep and goats, resp. The apparent vols. of distribution of ampicillin and sulbactam were similar in the two species. Mean body clearances of ampicillin were 0.69 L h-1 kg-1 in sheep and 0.72 L h-1 kg-1 in goats, and the body clearances of sulbactam were 0.38 and 0.38 L h-1 kg-1 in sheep and goats, resp. There were no significant differences between any of the pharmacokinetic parameters of ampicillin and sulbactam in the sheep and goats.

Carlsson PO, Sandler S, Jansson L. **Influence of the neurotoxin capsaicin on rat pancreatic islets in culture, and on the pancreatic islet blood flow of rats**. Eur J Pharmacol 1996;312(1):75-81.
The importance of peptidergic nerve fibres for the regulation of whole pancreatic and islet blood flow was studied by administration of the neurotoxin capsaicin. Administration of capsaicin induces an acute release and depletion of mainly substance P and calcitonin gene-related peptide from sensory nerve fibres. When given repeatedly to adult rats for several days, the neuropeptides are irreversibly depleted from the nerve endings. Depletion of substance P was confirmed by immunohistochemical stainings in the present study. A bolus dose of capsaicin (4 micrograms/kg body weight) reduced both whole pancreatic and islet blood flow in anesthetized rats, whereas repeated treatment with capsaicin led to an increase in both pancreatic and islet blood flow. In vitro experiments on isolated islets exposed to capsaicin (0.25 and 2.5 microM) for 4 days showed no effect on beta-cell function. We conclude that peptidergic nerves have an important role for the maintenance of basal vascular tone in both the endocrine and exocrine parts of the pancreas, and may thereby influence the regulation of insulin

secretion in rats.

Chalvet-Monfray K, Auger P, Belzunces LP, Fleche C, Sabatier P. **Modelling based method for pharmacokinetic hypotheses test**. Acta Biother 1996;44(3-4):335-48.
BIOSIS COPYRIGHT: BIOL ABS. The aim of this work is to propose methods to test mechanism of synergy of toxic agents in bees. A synergy between prochloraz, an imidazole fungicide, and deltamethrin, a pyrethroid insecticide, was demonstrated experimentally. The hypothesis is that prochloraz modifies the penetration or the metabolism of deltamethrin. This hypothesis is tested using a pharmacokinetic box model. A previous experimental work showed that bee instantaneous mortalities were higher, from the time t1 to the time t2 after spraying, in groups sprayed with deltamethrin at dose D0 in the presence of prochloraz (DELTA+P) than in those sprayed with deltamethrin alone at a dose a time as high (alphaDELTA). We postulate that accrued mortality is proportional to the cumulated internal deltamethrin (ID2). ID2 of treatment (DELTA+P) had to be greater than ID2 of treatment (alphaDELTA) during the period from t1 to t2 so that the hypothesis would be consistent with the experimental data. The limit, for which the hyp conceivable, is the ID2(alphaDELTA) = ID2(DELTA +P) curve. We study, in particular, the asymptotic behaviour of the limit curve when different parameters of the kinetic model tend to O or . These limits allow to verify quickly and easily whether a mechanism is conceivable or not. As the limits are calculated with algebraic values, the test can be used for other synergies.

Cunningham A, Klopman G, Rosenkranz HS. **The carcinogenicity of diethylstilbestrol: structural evidence for a non-genotoxic mechanism**. Arch Toxicol 1996;70(6):356-61.
An analysis of the structure of diethylstilbestrol (DES) indicates that neither DES nor any of its metabolites are potential mutagens. Moreover, the present analyses suggest (a) that the observed carcinogenic spectrum of DES reflects the activity of metabolic intermediates and (b) that the carcinogenicity of DES in mice is due to the presence of a 6 A geometric descriptor that appears to be related to an estrogen receptor.

Cunningham ML. **Role of increased DNA replication in the carcinogenic risk of nonmutagenic chemical carcinogens**. Mutat Res 1996;365(1-3):59-69.
BIOSIS COPYRIGHT: BIOL ABS. DNA replication is not an error-free process; therefore induction of cell proliferation with the requisite increase in DNA replication may be an important mechanism by which carcinogenesis can be induced by chemicals. Data presented in this overview indicate a positive association between increased cell proliferation and carcinogenesis, and illustrate the value of performing mechanistic studies such as cell proliferation assays in conjunction with short-term tests to further investigate the results of cancer bioassays. Whereas chemically-induced cell proliferation per se may not be sufficient to induce carcinogenesis, it creates a favorable environment for tumor development. There are two types of chemically-induced cell proliferation, mitogenic and cytotoxic, and they have different consequences regarding the mechanism of carcinogenesis of a chemical. Mitogenic chemical such as phenobarbital, oxazepam, and the peroxisome proliferating agents exert a short-term cell proliferative response that may exert its primary effect in carcinogenesis at the promotion stages. It is not clear at what stage(s) cytotoxic agents such as methapyrilene, alpha2u-globulin inducers or saccharin production of chemical specific pleiotropic effects that may contribute to the carcinogenicity of a chemical. It is clear that mechanistic studies performed to understand the relationship of sex, species

and dose in rodent carcinogenicity assays of chemicals is critical for the extrapolation of such data for human health assessments.

El-Masri HA, Constan AA, Ramsdell HS, Yang R. **Physiologically based pharmacodynamic modeling of an interaction threshold between trichloroethylene and 1,1-dichloroethylene in Fischer 344 rats**. Toxicol Appl Pharmacol 1996;141(1):124-32.
BIOSIS COPYRIGHT: BIOL ABS. Physiologically based pharmacokinetic modeling (PBPK) and gas uptake experiments have been used by researchers to demonstrate the competitive inhibition mechanism between trichloroethylene (TCE) and 1,1-dichloroethylene (DCE). Expanding on their work, we showed that this pharmacokinetic interaction was absent at levels of 100 ppm or less of either chemical in gas uptake systems. In this study, we further illustrate the presence of such an interaction threshold at the pharmacodynamic level by examining the interaction effect of either chemical on the other's ability to bind and deplete hepatic glutathione (GSH) in Fischer 344 rats. However, at this end point, the pharmacodynamic interaction is complicated by the ability of the liver to resynthesize GSH in response to its depletion. To quantitatively resolve the interaction effects on GSH content from the resynthesis effects, physiologically based pharmacodynamic (PBPD) modeling is applied. Initially, the PBPD model description of hepatic GSH kinetics was calibrated against previously published data and by gas uptake experiments conducted in our laboratory. Then, the model was used to determine the duration of the gas uptake exposure experiments by identifying the critical time point at which hepatic GSH is at a minimum in response to both chemicals. Subsequently, gas uptake experiments were designed following the PBPK/PD model predictions. In these model-directed experiments, DCE was the only chemical capable of significantly depleting hepatic GSH. The application of TCE to the rats at concentrations higher than 100 ppm obstructed the ability of DCE to deplete hepatic GSH. Since the metabolites of DCE bind to hepatic GSH, this obstruction signaled the presence of metabolic inhibition by TCE. However, TCE, at concentrations less than 100 ppm, was not effective in inhibiting DCE from significantly depleting hepatic GSH. The same observations were made when the ability of DCE to cause hepatic injury, as measured by aspartate aminotransferase serum activity, was assessed. Both conclusions validated the previous findings of the presence of the interaction threshold at the pharmacokinetic level.

El-Masri HA, Thomas RS, Sabados GR, Phillips JK, Constan AA, Benjamin SA, Andersen ME, Mehendale HM, Yang RS. **Physiologically based pharmacokinetic/pharmacodynamic modeling of the toxicologic interaction between carbon tetrachloride and Kepone**. Arch Toxicol 1996;70 (11):704-13.
Carbon tetrachloride (CCl4) lethality in Sprague-Dawley rats is greatly amplified by pretreatment of Kepone (decachlorooctahydro-1,3,2-metheno-2H-cyclobuta[cd] pentalen-2-one). The increase in lethality was attributed to the obstruction of liver regenerative processes. These processes are essential for restoring the liver to its full functional capacity following injury by CCl4. Based on the available mechanistic information on Kepone/CCl4 interaction, a physiologically based pharmacokinetic/ pharmacodynamic (PBPK/PD) model was constructed where the following effects of Kepone on CCl4 toxicity are incorporated: (1) inhibition of mitosis; (2) reduction of repair mechanism of hepatocellular injury; (3) suppression of phagocytosis. The PBPK/PD model provided computer simulation consistent with previously published time-course results of hepatotoxicity (i.e., pyknotic, injured and mitotic cells)

of CCl4 with or without Kepone. As a further verification of this model, the computer simulations were also consistent with exhalation kinetic data for rats injected with different intraperitoneal (i.p.) doses of CCl4 in our laboratory. Subsequently, the PBPK/PD model, coupled with Monte Carlo simulation, was used to predict lethalities of rats treated with CCl4 alone and CCl4 in combination with Kepone. The experimental lethality studies performed in our laboratories were as follows: Sprague-Dawley rats were given either control diet or diet containing 10 ppm Kepone for 15 days. On day 16, rats in the Kepone treated group were given i.p. doses of 0, 10, 50, and 100 microliters/kg CCl4 (n = 9) while control rats were exposed to 0, 100, 1000, 3000, and 6000 microliters/kg CCl4 (n = 9). Lethality was observed at the 1000 (1/9), 3000 (4/9), and 6000 (8/9) microliters/kg doses for the control group and at the 50 (4/9) and 100 (8/9) microliters/kg for the treated group. Based on Monte Carlo simulation, which was used to run electronically 1000 lethality experiments for each dosing situation, the LD50 estimates for CCl4 toxicity with and without Kepone pretreatment were 47 and 2890 microliters/kg, respectively. Monte Carlo simulation coupled with the PBPK/PD model produced lethality rates which were not significantly different from the observed mortality, with the exception of CCl4 at very high doses (e.g., 6000 microliters/kg, p = 0.014). Deviation at very high doses of the predicted mortality from the observed may be attributed to extrahepatic systemic toxicities of CCl4, or solvent effects on tissues at high concentrations, which were not presently included in the model. Our modeling and experimental results verified the earlier findings of Mehendale (1990) for the 67-fold amplification of CCl4 lethality in the presence of Kepone. However, much of this amplification of CCl4 lethality with Kepone pretreatment was probably due to pharmacokinetic factors, because when target tissue dose (i.e., model estimated amount of Ccl4 metabolites) was used to evaluate lethality, this amplification was reduced to 4-fold.

Evans MV, Simmons JE. **Physiologically based pharmacokinetic estimated metabolic constants and hepatotoxicity of carbon tetrachloride after methanol pretreatment in rats**. Toxicol Appl Pharmacol 1996;140(2):245-53.

A single 6-hr exposure to inhaled methanol (MeOH) has been shown to enhance carbon tetrachloride (CCl4) hepatotoxicity. The objective of the present study was to use gas uptake data and the development of a physiologically based pharmacokinetic model (PBPK) to determine in vivo changes in CCl4 metabolism resulting from MeOH pretreatment. Adult male F344 rats (167-197 g) were exposed to 10,000 ppm MeOH (constant concentration) via inhalation for 6 hr. Individual rats were exposed using gas uptake techniques to CCl4 alone or to CCl4 either 24 or 48 hr after initiation of MeOH pretreatment. The following initial concentrations were used for CCl4: 0, 25, 100, 250, and 1000 ppm with exposures lasting 6 hr. Vmax (metabolic rate) was estimated from gas uptake data and Km (Michaelis constant) was assumed constant after methanol pretreatment. For CCl4 alone, Vmax was 0.11 mg/hr (Vmaxc = 0.37 mg/hr/kg) and Km was 1.3 mg/liter. Vmax was 0.48 mg/hr (Vmaxc = 1.6 mg/hr/kg) for the 24-hr MeOH + CCl4 group and Vmax was 0.18 mg/hr (Vmaxc = 0.6 mg/hr/kg) for the 48-hr MeOH + CCl4 group. For CCl4 alone, serum markers of hepatotoxicity alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH) were increased significantly only at 1000 ppm CCl4. Both serum markers of hepatotoxicity in the 24-hr MeOH + CCl4 group increased as a function of CCl4 concentration when compared with 0 ppm CCl4 controls. The maximum increase occurred at 1000 ppm CCl4, where ALT and SDH increased by 392- and 286-fold, respectively. At 100, 250, and 1000 ppm CCl4, ALT and SDH values for the 24-hr MeOH + CCl4 groups were significantly increased relative to control (0 ppm CCl4), CCl4 alone, and 48-hr MeOH + CCl4. ALT and SDH levels in the 48-hr MeOH + CCl4 groups were not

statistically different from the respective CCl4 alone groups.

Friedberg T, Holler R, Lollmann B, Arand M, Oesch F. **The catalytic activity of the endoplasmic reticulum-resident protein microsomal epoxide hydrolase towards carcinogens is retained on inversion of its membrane topology**. Biochem J 1996;319(Pt 1):131-6.
Diol epoxides formed by the sequential action of cytochrome P-450 and the microsomal epoxide hydrolase (mEH) in the endoplasmic reticulum (ER) represent an important class of ultimate carcinogenic metabolites of polycyclic aromatic hydrocarbons. The role of the membrane orientation of cytochrome P-450 and mEH relative to each other in this catalytic cascade is not known. Cytochrome P-450 is known to have a type I topology. According to the algorithm of Hartman, Rapoport and Lodish [(1989) Proc. Natl. Acad. Sci. U.S.A. 86, 5786-5790], which allows the prediction of the membrane topology of proteins, mEH should adopt a type II membrane topology. Experimentally, mEH membrane topology has been disputed. Here we demonstrate that, in contrast with the theoretical prediction, the rat mEH has exclusively a type I membrane topology. Moreover we show that this topology can be inverted without affecting the catalytic activity of mEH. Our conclusions are supported by the observation that two mEH constructs (mEHg1 and mEHg2), containing engineered potential glycosylation sites at two separate locations after the C-terminal site of the membrane anchor, were not glycosylated in fibroblasts. However, changing the net charge at the N-terminus of these engineered mEH proteins by +3 resulted in proteins (++mEHg1 and ++mEHg2) that became glycosylated and consequently had a type II topology. The sensitivity of these glycosylated proteins to endoglycosidase H indicated that, like the native mEH, they are still retained in the ER. The engineered mEH proteins were integrated into membranes as they were resistant to alkaline extraction. Interestingly, an insect mEH with a charge distribution in its N-terminus similar to ++mEHg1 has recently been isolated. This enzyme might well display a type II topology instead of the type I topology of the rat mEH. Importantly, mEHg1, having the natural cytosolic orientation, as well as ++mEHg1, having an artificial huminal orientation, displayed rather similar substrate turnovers for the mutagenic metabolite benzo[a]pyrene 4,5-oxide. To our knowledge this is the first report demonstrating that topological inversion of a protein within the membrane of the ER has only a moderate effect on its enzymic activity, despite differences in folding pathways and redox environments on each side of the membrane. This observation represents an important step in the evaluation of the influence of mEH membrane orientation in the cascade of events leading to the formation of ultimate carcinogenic metabolites, and for studying the general importance of metabolic channelling on the surface of membranes.

Fujimiya T, Li Y, Uemura K, Ohbora Y, Komura S. **Noncompetitive-like inhibition of ethanol elimination by cyanamide treatment: pharmacokinetic study**. Alcohol:Clin Exp Res 1996;20(9 Suppl):278a-283a.
CBAC COPYRIGHT: CHEM ABS The effect of acetaldehyde accumulation is of interest in medico-legal practice in Japan. We examd. the pharmacokinetic mechanism of the inhibition of ethanol metab. by cyanamide, an inhibitor of mitochondrial aldehyde dehydrogenase. An ethanol soln. (0.25-2.0 g/kg body wt.) was injected i.v. into male rabbits with or without administration of cyanamide. Cyanamide was injected i.p. (25 mg/kg body wt.) to the cyanamide-treated group 2 h before ethanol injection. Blood ethanol and acetaldehyde concns. were measured periodically by head-space gas chromatog. The MULTI (RUNGE) computer program was applied for the pharmacokinetic anal. One- or two-compartment open

models with Michaelis-Menten elimination kinetics were used for simultaneous multi-line fitting. The ethanol elimination rate decreased after cyanamide treatment. The border-point concn. between pseudolinear and curvilinear phases was not affected by cyanamide treatment. The estd. Vmax value decreased by cyanamide treatment, whereas the Km value did not change. Our results correspond to a noncompetitive-like inhibition of ethanol metab. Km is related to the border point between pseudolinear and curvilinear phases. Thus, our findings in the blood ethanol concn.-time curve suggest adequate curve-fitting. The product, or competitive, inhibition of alc. dehydrogenase by acetaldehyde had been reported in enzymol. study. The pharmacokinetic manner of inhibition in vivo was different from the enzymol. mechanism in vitro. Other metabolic factors related to ethanol metab. are thought to be more important than acetaldehyde accumulation itself.

Furihata C, Oka M, Amin S, Krzeminski J, Weisburger JH, Kobayashi K, Tatematsu M. **Effect of 2-chloro-4-methylthiobutanoic acid in a rapid bioassay for gastric carcinogens**. Cancer Lett 1996; 108 (1):129-35.
2-Chloro-4-methylthiobutanoic acid (CMBA, a mutagen from Japanese salted fish) at 15-500 mg/kg body weight induced several-fold increase in replicative DNA synthesis (RDS) ($P < 0.05$) after 80 min and 17 h, equivocal unscheduled DNA synthesis (UDS) after 80 min and necrosis 80 min after its administration in the stomach pyloric mucosa of F344 and ACI male rats. A positive control, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG, 50 mg/kg body weight), induced RDS, UDS and erosion. However, the negative control L-methionine (500 mg/kg body weight) did not display any effect. The results suggest possible tumor-initiating and -promoting activity of CMBA but at a lower potency than that of MNNG.

Gobburu JV, Chen EP. **Artificial neural networks as a novel approach to integrated pharmacokinetic-pharmacodynamic analysis**. J Pharm Sci 1996 May; 85(May):505-10.
IPA COPYRIGHT: ASHP A novel model-independent approach to analyze pharmacokinetic/ pharmacodynamic data using artificial neural networks (ANNs) is presented. ANNs are versatile computational tools that possess the attributes of adaptive learning and self-organization. ANNs of one architecture are shown to be flexible enough to accurately predict pharmacodynamic profiles for a wide variety of pharmacokinetic/pharmacodynamic relationships. Also, an example is given of the ability of ANNs to accurately predict pharmacodynamic profiles without requiring any information regarding the active metabolite. Because structural details are not required, ANNs exhibit a clear advantage over conventional model-dependent methods.

Goelzer P, Janzowski C, Pool-Zobel BL, Eisenbrand G. **(E)-2-hexenal-induced DNA damage and formation of cyclic 1,N2-(1,3-propano)-2'-deoxyguanosine adducts in mammalian cells**. Chem Res Toxicol 1996;9(7):1207-13.
BIOSIS COPYRIGHT: BIOL ABS. (E)-2-Hexenal (hexenal), a natural flavor compound, acts as directly genotoxic agent and forms cyclic 1,N2-propano adducts with deoxyguanosine. Formation of this adduct in isolated DNA and in cells was studied with a modified 32P-postlabeling procedure including HPLC separation, nuclease P1 enrichment, two-dimensional TLC of adducted nucleotide bisphosphates on PEI-cellulose, and quantification of adduct spots by liquid scintillation counting. Adduct formation with the more reactive crotonaldehyde was included for comparison. Synthesized adducted dG-3'-phosphates served as external standards for identification and quantification. In calf thymus DNA, hexenal (0.2

mM) shows a time dependent formation of adducts, yielding 1.55 pmol/mumol of DNA at 5 h incubation. With crotonaldehyde (0.2 mM) the adduct rate was about 10-fold higher. Hexenal also generated 1,N2-propano-dG adducts in the human lymphoblastoid Namalva cell line (0.2 mM, 1 h, 86 fmol/mumol of DNA) and in primary rat colon mucosa cells (0.4 mM, 30 min, 50 fmol/ymol of DNA). In primary colon mucosa cells from rats and humans, hexenal and crotonaldehyde (0.4 mM, 30 min) induced DNA damage, detected by single cell microgel electrophoresis (comet assay). In primary rat gastric mucosa cells, hexenal was only weakly active, inducing detectable DNA damage in 20% of cells at 0.8 mM concentration. In contrast, primary mucosa cells from rat esophagus were as sensitive as colon cells. After single oral application of hexenal to rats (up to 320 mg/kg body wt) DNA damage was not detectable in gastrointestinal mucosa. Analysis of hexenal in selected flavored foods revealed concentrations up to 14 ppm (0.14 mM) that are comparable to its natural occurrence in some fruits and vegetables (up to 30 ppm). Thus, the concentration range selected for the toxicological studies described here clearly is relevant: Hexenal, at concentrations found in food, exerts genotoxic effects in cells from rat and human gastrointestinal tract.

Harmon CS, Ducote J, Xiong Y. **Thapsigargin induces rapid, transient growth inhibition and c-fos expression followed by sustained growth stimulation in mouse keratinocyte cultures**. J Invest Dermatol 1996;107(2):188-94.
Although the sesquiterpene lactone thapsigargin has been shown to possess hyperplastic and tumor-promoting activities when applied topically to mouse skin in vivo, the cellular mechanism(s) which underlie these effects are unclear. We show here that thapsigargin treatment of Primary mouse epidermal keratinocytes increased intracellular free Ca2+ concentration (Cai) in a concentration-dependent manner. Thapsigargin induced a rapid, transient elevation in keratinocyte Cai, in part due to the release of Ca2+ from intracellular stores. This response was followed by a sustained elevation in Ca2+, resulting entirely from calcium influx. Thapsigargin elicited a biphasic effect on keratinocyte DNA synthesis: a rapid inhibitory effect (50-60% inhibition at 4-8 h), followed by a very marked and sustained elevation. Prolonged treatment of keratinocytes with thapsigargin at relatively high concentrations resulted in cytotoxicity (inhibition of neutral red uptake). The rapid antiproliferative effect of thapsigargin was not associated with cytotoxicity, as determined by either neutral red uptake or by trypan blue exclusion, and was not blocked by pretreatment with Ro 31-7349, a selective inhibitor of protein kinase C. The rapid antiproliferative effect of thapsigargin was associated with rapid, transient activation of keratinocyte c-fos expression and rapid inhibition of total protein synthesis. Taken together, these findings raise the possibility that the hyperplastic and tumor-promoting activities of thapsigargin on epidermis in vivo result from direct keratinocyte growth stimulation as a consequence of a prolonged elevation in levels of Cai.

Hu X, Benson PJ, Srivastava SK, Mack LM, Xia H, Gupta V, Zaren HA, Singh SV. **Glutathione S-transferases of female A/J mouse liver and forestomach and their differential induction by anti-carcinogenic organosulfides from garlic**. Arch Biochem Biophys 1996;336(2):199-214.
This study characterizes glutathione (GSH) S-transferase (GST) isoenzymes of the liver and forestomach of the female A/J mouse and compares their specificities in catalyzing the conjugation of GSH with 7beta,8alpha-dihydroxy-9alpha,10alpha-oxy-7,8,9, 10-tetrahydrobenzo[a] pyrene (anti-BPDE), the ultimate carcinogenic metabolite of benzo[a]pyrene (BP). The GST activity in female A/J

mouse liver was expressed by a minimum of seven isoenzymes which arose from different homo- or heterodimeric combinations of at least two alpha class (designated as alpha1 and alpha4), four &mgr; class (&mgr;1 to &mgr;4), and one pi class GST subunit. The GST isoenzyme composition of A/J mouse forestomach appeared to be different from that of the liver. For example, while GST isoenzymes containing &mgr;3 and &mgr;4 type subunits were selectively expressed in the liver, an alpha class heterodimeric GST isoenzyme (containing alpha2 and alpha3 subunits) was expressed in the forestomach but could not be detected in the liver. The (+)-anti-BPDE appeared to be a better substrate than the (-)-enantiomer for all GSTs, except for isoenzymes containing the alpha4 type GST subunit. The murine pi class GST isoenzyme displayed relativey higher specific activity toward (+)-anti-BPDE compared to other GSTs. The specific activities of mouse GSTs toward (+)-anti-BPDE were in the order of pi > &mgr; > alpha. These results suggest that the pi class GST isoenzyme may play an important role in providing protection against BP-induced cancer. Therefore, it seems logical to postulate that the ability of a chemoprotector to increase the expression of GST pi may be an important determinant of its effectiveness against BP-induced cancer. To test the validity of this contention, we have determined the effects on hepatic and forestomach GST isoenzyme/subunit expression of three naturally occurring organosulfides (OSCs) from garlic, which significantly differ in their effectiveness against BP-induced forestomach cancer. Treatment of mice with diallyl sulfide (DAS) and diallyl trisulfide (DATS), which are potent inhibitors of BP-induced fore-stomach cancer in mice, resulted in a significant increase in hepatic and forestomach GST activity toward anti-BPDE. On the contrary, this activity was not increased in either organ by dipropyl sulfide (DPS), which is ineffective against BP-induced forestomach cancer. The chemopreventive efficacy of these OSCs correlated with their ability to increase the expression of GST pi. For example, DAS treatment resulted in approximate increases of 1.7- and 2.2-fold in hepatic and forestomach GST pi expression, respectively, over the control. Treatment of mice with DATS, which is a relatively more potent inhibitor of BP-induced forestomach cancer than DAS, resulted in about 3.8- and 3.2-fold increases, respectively, in hepatic and forestomach GST pi expression over the control. On the contrary, the expression of hepatic and forestomach GST pi was increased only marginally (10-20%) upon DPS administration. In conclusion, the results of the present study suggest that induction of GST pi can be used as a bioassay for screening potential inhibitors of BP-induced cancer.

Hulten K, Rigo R, Gustafsson I, Engstrand L. **New pharmacokinetic in vitro model for studies of antibiotic activity against intracellular microorganisms**. Antimicrob Agents Chemother 1996;40(2) 2727-31.

CBAC COPYRIGHT: CHEM ABS The capacity for intracellular growth in an important survival strategy for a large group of common pathogens. Helicobacter pylori, the etiol. agent for gastritis and duodenal ulcer, has been shown by both in vivo and in vitro studies to have the capacity to invade epithelial cells. In vitro models are used to study the effect of antibiotics on microorganisms. Most investigations are performed in broth culture or on agar plates, but kinetic models for bacteria in broth have been described. The authors present a new, kinetic model adapted for intracellular pathogens. A glass chamber, with a metal rack fitting Falcon cell culture inserts, was connected to a pump by rubber tubes. Different tube diams. and pump speeds were evaluated, and the assay was designed to mimic the half-lives of the antibiotics in vivo, i.e., 11.5 h for azithromycin, 5 h for clarithromycin, and 1 h for amoxicillin. Monolayers of HEp-2 cells were grown in the inserts for 2 days, after which H. pylori (clin.

strain 88-23), was added to the system. Internalization was allowed for 12 h, and extracellular H. pylori cells were eradicated with gentamicin. The inserts were moved to the glass chamber, contg. medium with 12.5 mg of either amoxicillin or azithromycin per L or 2.4 mg of clarithromycin per L. This represents 12.5, 50, and 80 times the extracellular min. bactericidal concn. value, resp. Samples were taken at 0, 2, 4, 6, 8, and 24 h. The HEp-2 cells were lysed, and intracellular bacteria were counted by plating. Inserts with infected cells grown in drug-free medium were included as controls for each time interval. A 3-log10 redn. of H. pylori was achieved in the expts. with azithromycin, and a 4-log10 redn. was achieved in the clarithromycin expts., while no intracellular effect was seen when amoxicillin was used. The antibiotic concns. at the sampling intervals were 12.5, 3.1, 0.8, 0.2, 0.05, and 0 mg/L for amoxicillin; antibiotic concns. at the sampling intervals were 12.5, 3.1, 0.8, 0.2, 0.05, and 0 mg/L for amoxicillin; antibiotic concns. at the sampling intervals were 12.5, 3.1, 1, 0.8, 0.2, 0.05, and 0 mg/L for amoxicillin; was used. The antibiotic concns. at the sampling intervals were 12.5, 3.1, 0.8, 0.2, 0.05, and 0 mg/L for amoxicillin; 12.5, 11.5, 10,9, 8, and 3 mg/L for anitazithromycin;d 2.4 1.8, 1.4, 1, 0.8 and 0 mg/L for clarithromycin. This new model for pharmacokinetic studies proves a useful too, with application for clarithromycin. This new mode for pharmacokinetic studies proves a useful tool, with application for broad range microorganisms.

Ientile R, Picciurro V, Pedale S, Nucci C, Malecka B, Nistico G, Macainoe S. **Nitric oxide enhances amino acid release from immature chick embryo retina**. Neurosci Lett 1996;219(2):79-82.
CBAC COPYRIGHT: CHEM ABS Nitric oxide (NO) was investigated for its ability to induce amino acid release from immature chick retina. The prodn. of endogenous NO by activation of NO synthase after stimulation of N-methyl-D-aspartate (NMDA) subtype of glutamate receptor caused a significant increase in basal release of gamma-aminobutyric acid (GABA) and glutamine, whereas a more modest increase in the glutamate release was also obsd. The exposure of chick retina from 9-day old embryos to NO-generating compds., S-nitroso-N-acetylpenicillamine (SNAP) and sodium nitroprusside (SNP) produced a dose dependent increase in GABA, glutamine, and glutamate release. This effect was reduced by about 80% by Hb. These results indicate that NO has a stimulatory effect on amino acid release from chick embryo immature retina. However, this effect does not appear to involve a cGMP-related mechanism because 8-bromo-cGMP, a stable analog of cGMP, failed to affect spontaneous amino acid release and because zaprinast did not enhance NMDA-stimulated release. In conclusion, the authors' present observations may account for a role of NMDA-mediated events in the biochem. maturation under depolarizing conditions.

International Expert Panel Carcinogen Risk Assessment. **The use of mechanistic data in the risk assessments of ten chemicals an introduction to the chemical-specific reveiws** . Pharmacol Ther 1996;71(1-2):1-5.
BIOSIS COPYRIGHT: BIOL ABS. RRM Journal article human rodent carcinogenesis exposure levels the international expert panel on carcinogen risk assessment of the american health foundation mathematical data analytical method.

Iwatsubo T, Hirota N, Ooie T, Suzuki H, Sugiyama Y. **Prediction of in vivo drug disposition from in vitro data based on physiological pharmacokinetics**. J Protein Chem 1996 May;17:273-310.
IPA COPYRIGHT: ASHP A review of several successful attempts to predict in vivo metabolic clearances in experimental animals and humans from in vitro biochemical parameters such as plasma

protein binding and hepatic metabolism, based on anatomically and physiologically realistic pharmacokinetic models, is presented, including a method proposed to overcome errors resulting from inter-individual differences by applying the concept of a scaling factor, and factors that should be considered in any in vitro/in vivo scaling.

Jackson HC, Biggadike K, Mckilligin E, Kinsman OS, Queener SF, Lane A, Smith JE. **6,7-disubstituted 2,4-diaminopteridines: novel inhibitors of Pneumocystis carinii and Toxoplasma gondii dihydrofolate reductase**. Antimicrob Agents Chemother 1996;40(6):1371-5.
Four novel, disubstituted diaminopteridines have been identified which antagonize the uptake of a folate precursor (para-aminobenzoic acid) by rat-derived Pneumocystis carinii maintained in short-term axenic culture at concentrations ranging from 4.5 to 26 microM. The compounds were at least 10 to 100 times more active than trimethoprim in this assay. None of these entities exhibited toxicity to mammalian cell lines at < 100 microM. The same structures also caused significant inhibition of Toxoplasma gondii tachyzoite replication within Madin-Darby bovine kidney cells at concentrations ranging from 0.1 to 10 microM. Three of the structures (GR92754, AH10639, and AH2504) were at least an order of magnitude more potent than the standard anti-T. gondii agent, pyrimethamine. All three entities were also significantly more potent and selective than pyrimethamine as inhibitors of T. gondii dihydrofolate reductase (DHFR), with 50% inhibitory concentrations within the range of 0.018 to 0.033 microM. One of these compounds, 6,7-dibutyl-2,4-diaminopteridine (GR92754), was also a potent and selective inhibitor of P. carinii DHFR (50% inhibitory concentration, 0.082 microM). GR92754 is the first DHFR inhibitor described that exhibits greater potency, selectivity, and intracellular activity against both organisms than any of the DHFR agents used clinically, namely, trimethoprim, pyrimethamine, and trimetrexate. This information could provide the starting point for examination of the pharmacokinetic and therapeutic potential of GR92754 and related chemical entities with animal models.

Johanson G, Filser JG. **PBPK model for butadiene metabolism to epoxides: quantitative species differences in metabolism**. Toxicology 1996;113(1-3):40-7.
We have developed a physiologically based pharmacokinetic (PBPK) model for 1,3-butadiene (BD) and its first reactive metabolite 1,2-epoxybutene-3 (EB). This model contrasts with other published ones, in that it incorporates three important features: (I) reduced alveolar ventilation, based on experimental observations on a number of vapors and gases; (II) intrahepatic first-pass hydrolysis of EB, based on experimental observations with BD-EB.

Kaltenbach G, Leveque D, Peter JD, Salmon J, Elkhaili H, Cavalier A, Salmon Y, Monteil H, Jehl F. **Pharmacokinetic interaction between itraconazole and rifampin in Yucatan miniature pigs**. Antimicrob Agents Chemother 1996;40(9):2043-6.
The objective of this study was to examine the effects of rifampin on itraconazole pharmacokinetics, at steady state, in three Yucatan miniature pigs. Daily for 3 weeks, the pigs received 200 mg of itraconazole orally at the beginning of each meal, and for the following 2 weeks they received itraconazole orally combined with intravenous administration of rifampin at 10 mg/kg/day. Coadministration of rifampin resulted in an 18-fold decrease in the maximum concentration of itraconazole in serum, from 113.0 (standard deviation [SD] 17.2) to 6.2 (SD, 3.9) ng/ml and a 22-fold decrease in the area under the concentration-time curve, from 1,652.7 (SD, 297.7) to 75.6 (SD, 30.0) ng. h/ml. The active metabolite of itraconazole, hydroxyitraconazole, was undetectable. This study

demonstrates that rifampin affects itraconazole kinetics considerably at steady state in this miniature-pig model, probably by inducing hepatic metabolism of itraconazole.

Kohn MC, Melnick RL. **Effects of the structure of a toxicokinetic model of butadiene inhalation exposure on computed production of carcinogenic intermediates**. Toxicology 1996;113(1-3):31-9. A flow-limited physiologically based toxicokinetic model was constructed for uptake, metabolism, and clearance of butadiene (BD) and its principal metabolite 1,2-epoxy-3-butene (EB), using physiological and biochemical parameters from the literature where available. The model includes compartments for blood, liver, lung, fat, GI tract, other rapidly perfused tissues, and slowly perfused tissues. The blood was distributed among compartments for arterial plus venous blood and subcompartments for vascular spaces associated with each of the tissue compartments. The lung contained a subcompartment for the alveolar space. Metabolic activation of BD by cytochrome P450-catalyzed epoxidation was modeled as occurring in liver, lung, and the rapidly perfused tissue compartments. The detoxication of EB catalyzed by epoxide hydrolase and glutathione S-transferase (GST) was modeled as occurring in liver, lung, and the rapidly perfused tissues compartments and by blood GST activity. The model also includes depletion of glutathione (GSH) by GST-catalyzed conjugation of EB and 3-butene-1,2-diol and resynthesis of GSH from cysteine. Values of biochemical parameters that were unavailable in the literature were estimated by iteratively reweighted least squares optimization to reproduce data for uptake of BD and EB by rats and mice in closed chambers. The resulting model also reproduced the depletion of GSH in liver and lung in flow-through systems. It reproduced the concentrations of expired EB produced from BD in closed chambers but overpredicted separately measured blood EB concentrations in flow-through systems, indicating an inconsistency between these two experiments that cannot be resolved by this model or an inadequacy in the model. Equilibration of chamber gases with the alveolar space and alveolar gas with lung capillary blood results in much less dilution of the inhaled gas in the blood compared with the predictions of models in which chamber gas equilibrates.

Kortenkamp A, Casadevall M, Da Cruz Fresco P. **The reductive conversion of the carcinogen chromium (VI) and its role in the formation of DNA lesions**. Ann Clin Lab Sci 1996;26(2):160-75. The potential of Cr(VI), in combination with glutathione (GSH) or ascorbate (AsA) to induce apurinic/apyrimidinic sites (AP-sites) and single strand breaks (SSB) in isolated deoxyribonucleic acid (DNA) was investigated. The observation that both lesions were formed with equal probability and followed a similar time course suggests that they might arise from attack of a reactive species at C4' of the DNA sugar moeity. This idea is further substantiated by the finding that malondialdehyde-like products are released in chromate/GSH- and chromate/AsA-treated DNA. The generation of AP-sites and SSB was dependent on molecular oxygen and could be suppressed by the addition of catalase. Our results rule out hydroxyl radicals as the DNA damaging species. Furthermore, Cr(V), an intermediate formed during reaction with GSH or AsA, is not directly involved in the generation of DNA damage, unless activated by molecular oxygen. Our findings indicate that a superoxo- or peroxo-complex involving Cr(V) or Cr (IV) might be the species responsible for DNA damage. Evidence is presented that the DNA lesions arising from chromate/AsA have the potential to cause gene mutations.

Krishnan K, Haddad S, Pelekis M. **A simple index for representing the discrepancy between simulations of physiological pharmacokinetic models and experimental data**. Toxicol Ind Health 1995;11(4):413-21.

An approach was presented for expressing and communicating in an objective manner the overall percent difference between physiologically based pharmacokinetic (PBPK) model simulations and data collected from experiments. The root mean square of the difference between the individual simulated and experimental value for each sampling point in a time course curve was determined and divided by the root mean square of the experimental values. The numerical values thus obtained served as discrepancy measures for several data sets. Values obtained for several sets were combined on the basis of a weighting proportional to the number of data points in each set. This was the method used to build a consolidated discrepancy index from several experiments. By averaging each of these an overall discrepancy index was obtained, referred to as the PBPK index. The final index was felt to reflect the overall, weighted average percent difference between the a-priori PBPK model simulations and the real data obtained from actual experimentation. To serve as an example of the usefulness of the index, the method was used with previously gathered data on dichloromethane (75092) pharmacokinetics in humans with both experimental and simulated data. The authors conclude that using this type of quantitative method may assist in removing the ambiguity in communicating the degree of concordance or discrepancy between PBPK model simulations and experimental data.

Kuipers ME, Swart PJ, Schutten M, Smit C, Proost JH, Osterhaus AD, Meijer DK. **Pharmacokinetics and anti-HIV-1 efficacy of negatively charged human serum albumins in mice**. Antiviral Res 1997; 33(2):99-108.
CBAC COPYRIGHT: CHEM ABS Neg. charged albumins (NCAs, with the prototypes succinylated human serum albumin (Suc-HSA) and aconitylated human serum albumin (Aco-HSA)), modified proteins with a potent anti-human immunodeficiency virus type 1 (anti-HIV-1) activity in vitro, were studied for their pharmacokinetic behavior in mice and their in vivo anti-HIV-1 efficacy in an HIV-1 infection model in mice. In contrast to the satn. kinetics found in rats, i.v. injections of 10-300 mg/kg for both NCAs showed a linear correlation between the area under the curve (AUC) and the dose. The elimination t1/2 was 25 and 30 min for Suc-HSA and Aco-HSA, resp. Preinjections of an excess of formaldehyde-treated albumin (Form-HSA) resulted in plasma levels that were 3- and 4-fold higher for Aco-HSA and Suc-HSA, resp. These data indicate that elimination is at least partly (scavenger) receptor-mediated. Organ distribution studies 10 min after injection showed an accumulation in liver (Suc-HSA 17.3% of the dose; Aco-HSA 20.9%) and lungs (Suc-HSA 12.7%; Aco-HSA 16.0). I.p. injection of 300 mg/kg Suc-HSA resulted in a final bioavailability of about 0.45. Suc-HSA was also evaluated for its in vivo neutralizing capacity in a human-to-mouse chimeric model for HIV-1 infection. I.p. injections of 300 and 3 mg/kg Suc-HSA, given 15-30 min before the mice were challenged with the virus, sufficed to protect these mice against infection with the HIV-1 IIIB strain.

Kuo EA, Hambleton PT, Kay DP, Evans PL, Matharu SS, Little E, McDowall N, Jones CB, Hedgecock CJ R, et al. **Synthesis, structure-sctivity relationships, and pharmacokinetic properties of dihydroorotate dehydrogenase inhibitors: 2-cyano-3-cyclopropyl-3-hydroxy- N-[3'-methyl-4'-(trifluoromethyl) phenyl]propenamide and related compounds**. J Med Chem 1996;39(23):4608-21.

Landoni MF, Lees P. **Pharmacokinetics and pharmacodynamics of ketoprofen enantiomers in the horse**. J Vet Pharmacol Ther 1996;19(6):466-74.
CBAC COPYRIGHT: CHEM ABS Pharmacokinetic and pharmacodynamic parameters were

established for enantiomers of the non-steroidal anti-inflammatory drug (NSAID) ketoprofen (KTP), each administered sep. at a dose level of 1.1 mg/kg to a group of six New Forest geldings, in a three-period cross-over study using a tissue cage model of inflammation. For both S(+)- and R(-)-KTP, penetration into tissue cage fluid (transudate) and inflamed tissue cage fluid (exudate) was rapid, and clearances from exudate and transudate were much slower than from plasma. AUC values were, therefore, higher for exudate and, to a lesser degree, transudate than for plasma. Unidirectional chiral inversion of R(-)- to S(+)-KTP was demonstrated. Administration of both enantiomers produced marked, time-dependent inhibition of synthesis of serum thromboxane B2 and exudate prostaglandin E2, indicating non-selective inhibition of cyclo-oxygenase (COX) isoenzymes COX-1 and COX-2 resp. Administration of both enantiomers also produced partial inhibition of beta-glucuronidase release into inflammatory exudate and of bradykinin-induced skin edema. It is suggested that, although S(+)-KTP is generally regarded as the eutomer, R(-)-KTP was probably at least as active in inhibiting bradykinin swelling. Pharmacokinetic/pharmacodynamic (PK/PD) modeling of the data could not be undertaken following R(-)-KTP administration because of chiral inversion to S(+)-KTP, but pharmacodynamic parameters, Emax, EC50, N, keO and t1/2(keO), were detd. for S(+)-KTP using the sigmoidal Emax equation. PK/DP modeling provided a novel means of comparing and quantifying several biol. effects of KTP and of investigating its mechanisms of action.

Leahy D, Arundel P, Blakey G, Rowland M. **Physiologic based pharmacokinetic modeling and QSAR**. Bioact Compd Des 1996;147-51.
CBAC COPYRIGHT: CHEM ABS A preliminary anal. of tissue pharmacokinetic data generated in rat for a series of 5-alkyl-5-Et barbituric acids is reported. The anal. partially supports the hypothesis that the whole animal pharmacokinetic profile can be simulated from a knowledge of physiol. parameters such as tissue vols. and blood flows, combined with the key solute dependent properties such as tissue partition coeffs. In addn., physiol. parameters are shown to be simply dependent on phys. properties such as lipophilicity.

Leavens TL, Bond JA. **Pharmacokinetic model describing the disposition of butadiene and styrene in mice**. Toxicology 1996;113(1-3):310-3.
Coexposure to 1,3-butadiene (BD) and styrene occurs in the workplace of many polymer industries. The reactive epoxide metabolites of both compounds are responsible for their genotoxicity. A physiologically based pharmacokinetic (PBPK) model was developed to describe the simultaneous disposition of BD and styrene in mice coexposed by inhalation. A model with one oxidative pathway and competition between BD and styrene was compared with a model with two oxidation pathways for both BD and styrene. The different PBPK models were used to simulate the observed rate of BD metabolism and blood concentration of styrene from 8-h inhalation exposures of mice to mixtures of BD and styrene. The model with two oxidative pathways more accurately simulated the observed inhibition of BD uptake in coexposed mice.

Lee YS, Choi JY, Park MK, Choi EM, Kasai H, Chung MH. **Induction of oh8Gua glycosylase in rat kidneys by potassium bromate (KBrO3), a renal oxidative carcinogen**. Mutat Res 1996;364(3):227-33.
It has been suggested that 8-hydroxyguanine (oh8Gua), a DNA adduct formed by active oxygens, impairs the maintenance of genetic integrity, oh8Gua glycosylase removes oh8Gua residues as a free

base from DNA strands. In E. coli, it has been demonstrated that oh8Gua glycosylase is induced in response to oxidative stress, but the oxidative inducibility in mammalian tissues has not yet been studied. In the present study, the inducibility of oh8Gua glycosylase was tested by comparing activity changes of this enzyme in the kidney and the liver of rats treated with potassium bromate (KBrO3). KBrO3 is known to cause oxidative damage to the kidney but not to other organs. With a single dose of KBrO3 (80 mg/kg, i.p.), activity in the kidney was found to increase significantly at 3 h compared to that at zero time. At 6 h, activity peaked, showing a 6-fold increase over that at zero time. Thereafter, it decreased and returned to its zero time level at 12 h. With increasing doses of KBrO3 (up to 160 mg/kg, i.p.), activity increased linearly with increased dosage, and over 40 mg/kg, i.p., activity increased to a level significantly higher than that in the control. In contrast to the time- and dose-dependent changes in activity in the kidney, no significant change was observed in the liver under the same conditions as above. These results show that oh8Gua glycosylase is also induced oxidatively in mammalian tissues. The induction in this tissue as well as in E. coli indicates that the adaptive response of this enzyme to oxidative stress is a general phenomenon in aerobic organisms and implies that the repair of oh8Gua residues in DNA is a process important for the survival of organisms in an aerobic environment.

Louis Anthony CJ. **Reassessing benzene risks using internal doses and Monte-Carlo uncertainty analysis**. Environ Health Perspect Suppl 1996;104(6):1413-29.
CBAC COPYRIGHT: CHEM ABS Human cancer risks from benzene have been estd. from epidermiol. data, with supporting evidence from animal bioassay data. This article reexamines the animal-based risk assessments using physiol. based pharmacokinetic (PBPK) models of benzene metab. in animals and humans. Internal doses (total benzene metabolites) from oral gavage expts. in mice are well predicted by the PBPK model. Both the data and the PBPK model outputs are also well described by a simple nonlinear (Michaelis-Menten) regression model, as previously used by A. J. Bailer and D. G. Hoel (1989). Refitting the multistage model family to internal doses changes the max.-likelihood est. (MLE) dose-response curve for mice from linear-quadratic to purely cubic, so that low-dose risk ests. are smaller than in previous risk assessments. In contrast to Bailer and Hoel's findings using interspecies dose conversion, the use of internal dose ests. for humans from a PBPK model reduces estd. human risks at low doses. Sensitivity analyses suggest that the finding of a nonlinear MLE dose-response curve at low doses is robust to changes in internal dose definitions and more consistent with epidemiol. data than earlier risk models. A Monte-Carlo uncertainty anal. based on max.-entropy probabilities and Bayesian conditioning is used to develop an entire probability distribution for the true but unknown dose-response function. This allows the probability of a pos. low-dose slope to be quantified: it is about 10%. An upper 95% confidence limit on the low-dose slope of excess risk is also obtained directly from the posterior distribution and is similar to previous q1* values. This approach suggests that the excess risk due to benzene exposure may be nonexistent (or even neg.) at sufficiently low doses. Two types of biol. information about benzene effects-pharmacokinetic and hematotoxic-are examd. to test the plausibility of this finding. A framework for incorporating causally relevant biol. information into benzene risk assessment is introduced, and it is shown that both pharmacokinetic and hematotoxic models appear to be consistent with the hypothesis that sufficiently low concns. of inhaled benzene do not create an excess risk.

Mann S, Droz PO, Vahter M. **A physiologically based pharmacokinetic model for arsenic exposure.**

II. Validation and application in humans. Toxicol Appl Pharmacol 1996;140(2):471-86.
A physiologically based pharmacokinetic model (PB-PK) for inorganic arsenic exposure in humans has been developed. This model is an extension of a PB-PK model for hamsters and rabbits, with adjustments for body weight, metabolic rates, and absorption rates. It describes the absorption, distribution, metabolism, and excretion of arsenate, arsenite (As(III)), methyl arsonate, and dimethyl arsinate, the four major metabolites of inorganic arsenic. The routes of intake considered are inhalation of arsenic dust and fumes and oral intake of arsenic via drinking water and food. The PB-PK model for the oral exposure route is validated using data on urinary excretion after repeated oral exposure to As (III) as well as after exposure to inorganic As via drinking water. Absorption by inhalation is validated using data on urinary excretion after occupational exposure to arsenic trioxide dust and fumes. In both cases, the model gives satisfactory results for urinary excretion of the four As metabolites. The PB-PK model is also used in the description of the effects on the kinetics of exposure via different routes and for the simulation of various realistic exposure scenarios.

Marchant CA, Combes RD. **Artificial intelligence: the use of computer methods in the prediction of metabolism and toxicity**. Bioact Compd Des 1996;153-62.
CBAC COPYRIGHT: CHEM ABS Computer methods for the prediction of metab. and toxicity provide one potential rapid and inexpensive alternative to animal experimentation. The DEREK, TOPKAT, CASE, COMPACT and HAZARDEXPERT systems use different approaches to predict toxicity on the basis of structure-activity relationships (SARs). Each of these systems has been used to predict the carcinogenicity of 44 chems. undergoing testing by the United States National Toxicol. Program (NTP). Comparison of their predictions with the exptl. outcome provides a useful indication of the relative predictive capabilities of each method. The META and METABOLEXPERT programs use a series of rules describing metabolic transformations to predict the metabolites of a compd. A major challenge for these systems is the discrimination between the large no. of metabolites which can potentially be formed and the limited no. which actually form in practice.

Meinertz JR, Stehly GR, Gingeric W. **Pharmacokinetics of benzocaine in rainbow trout (Oncorhynchus mykiss) after intraarterial dosing**. Aquaculture 1996;148(1):39-48.
CBAC COPYRIGHT: CHEM ABS The pharmacokinetics of benzocaine were analyzed in rainbow trout (Oncorhynchus mykiss) after intraarterial bolus administration of benzocaine at nominal concns. of 6 and 9 mg/kg. Distributive phase pharmacokinetic parameters were difficult to est. Benzocaine concns. at 2 min after dosing were highly variable and decreased rapidly within the first 10 min. Benzocaine concns. were near or below the quantitation limit 90 min after dosing. A three-compartment pharmacokinetic model best described benzocaine concns. in plasma. The apparent vol. of distribution at steady state and total body clearance increased with respect to dose. Since the mean residence time was similar at both dosages, the differences in the apparent vol. of distribution at steady state and total body clearance may have been the result of data variability or the degree of anesthesia rather than dose dependency. The model parameters indicated that distribution of benzocaine outside of the plasma was limited. Despite the initial rapid distribution and elimination of benzocaine from plasma, terminal phase benzocaine elimination was relatively slow (terminal elimination phase half-lives of 89 and 109 min).

Montecucco C, Schiavo G, Tugnoli V, De Grandis D. **Botulinum neurotoxins: mechanism of action and therapeutic applications**. Mol Med Today 1996;2(10):418-24.

Recent studies have led to the discovery of the molecular lesions in motor neurons caused by botulinum neurotoxins. These neurotoxins are metalloproteinases that enter the cytosol and very specifically cleave protein components of the neuroexocytosis apparatus. Consequently, acetylcholine cannot be released and the muscle is paralysed. For this reason, botulinum neurotoxins are increasingly being used to treat a variety of conditions where a functional paralysis of neuromuscular junctions is useful as therapy.

Nau H, Elmazar MM, Ruehl R, Thiel R, Sass JO. **All-trans-retinoyl-beta-glucuronide is a potent teratogen in the mouse because of extensive metabolism to all-trans-retinoic acid**. Teratology 1996;54(3):150-6.
BIOSIS COPYRIGHT: BIOL ABS. All-trans-retinoyl-beta-D-glucuronide (all-trans-RAG) is a water-soluble derivative of all-trans-retinoic acid (all-trans-RA) and has been characterized as an endogenous metabolite of vitamin A in rat bile and kidney. All-trans-RAG was previously demonstrated to be a major metabolite after application of all-trans-RA in several species (mouse, rat, rabbit, monkey); all-trans-RAG was described in these experiments to exhibit a very low placental transfer to the embryo. Because retinoid-like activity has been found after application of all-trans-RAG in vivo as well as in several in vitro systems, and because of its low placental transfer, this glycoconjugate appeared to be an interesting retinoid with possible therapeutic activity, but reduced teratogenicity. Here we investigated the teratogenic activity of all-trans-RAG in comparison to all-trans-RA in mice, and performed accompanying pharmacokinetic studies. Surprisingly, all-trans-RAG was more teratogenic than equimolar doses of all-trans-RA following subcutaneous application on day 11 of gestation in the mouse (20 mumol/kg body weight). Pharmacokinetic studies revealed that all-trans-RAG was extensively hydrolyzed to all-trans-RA and that the plasma area under the concentration-time curve (AUC) of all-trans-RA following all-trans-RAG application exceeded the plasma AUC value.

Nicholls-Grzemski FA, Burcham PC, Calder IC, Priestly BG. **Pretreatment with peroxisome proliferators protects mice against some but not all hepatotoxins**. Ann N Y Acad Sci 1996;804:742-4.

Nishii K, Kabarowski JH, Gibbons DL, Griffiths SD, Titley I, Wiedemann LM, Greaves MF. **ts BCR-ABL kinase activation confers increased resistance to genotoxic damage via cell cycle block**. Oncogene 1996;13(10):2225-34.
Using a temperature-sensitive mutant of the p210 BCR-ABL gene, transfected into a growth factor-dependent cell line (BaF3), we show that transient BCR-ABL kinase expression increases single cell and clonogenic resistance to apoptosis arising from genotoxic damage induced by ionizing radiation and VP-16/etoposide. This effect is achieved in the absence of any detectable changes in the levels of BCL-2, BAX or BCL-x proteins and is independent of proliferative, MAP kinase-dependent effects of BCR-ABL kinase. In contrast to parental cells that transiently arrest in G2 and then apoptose, p210 BaF3 cells show a pronounced and sustained G2 arrest following radiation coupled with enhanced phosphorylation of cdc2. A cell cycle block in early M phase induced by the mitotic spindle poison, nocodazole, does not provide protection from apoptosis. Reversal of G2 arrest by caffeine abolishes the protective effect of BCR-ABL kinase. These data provide further insight into the transforming properties of BCR-ABL and are relevant to the clinical intransigence of Ph-positive leukaemias.

Nishikawa M, Takakura Y, Hashida M. **Pharmacokinetic evaluation of polymeric carriers**. Adv Drug Delivery Rev 1996;21(2):135-55.
CBAC COPYRIGHT: CHEM ABS A review with 137 refs. The usefulness of polymeric carriers is evaluated by pharmacokinetic anal. of their in vivo disposition properties. The anal., based on a clearance concept, translates the properties into quant. pharmacokinetic parameters which can be compared with physiol. parameters such as plasma flow rate, glomerular filtration rate, and rate of fluid-phase endocytosis. Apparent hepatic uptake clearance and urinary excretion clearance are the important parameters detg. the in vivo distribution characteristics of macromols. The study using model macromols. has revealed that a macromol. having weak-anionic charge and mol. wt. larger than 40000 would be a good carrier for targeted delivery of covalently bound drugs. Carbohydrate receptor-glycosylated macromol. and antibody-antigen interactions are also pharmacokinetically analyzed by a pharmacokinetic model including a saturable process. Furthermore, the pharmacol. benefit of a drug-carrier conjugate is estd. by AUC values of free drug released from the carrier. These pharmacokinetic evaluations should make a significant contribution to a rational design of targetable polymeric carriers.

Nozaki Y, Ohta M, Chien YW. **Transmucosal controlled systemic delivery of isosorbide dinitrate: in vivo/in vitro correlation**. J Control Release 1997;43(2-3):105-14.
CBAC COPYRIGHT: CHEM ABS A bilayer track field-shaped transmucosal therapeutic system (TmTs) has been developed for the systemic delivery of therapeutic agents that are subject to an extensive pre-systemic clearance, using isosorbide dinitrate (ISDN) as the model drug. The transmucosal systemic delivery of ISDN was investigated by single gingival application of TmTs to beagle dogs. The cumulative absorption profiles and pharmacokinetic profiles of ISDN were monitored. Both were found to be directly correlated with the release profiles of ISDN detd. by in vitro dissoln. studies. An excellent (1:1) correlation at Level A was achieved when the dissoln. test was conducted in a medium of pH 6.8 with the paddle rotating at a rotation speed of 200 rpm. Furthermore, the transmucosal systemic delivery of ISDN from TmTs was obsd. to depend on the release rate of ISDN: as the release rate decreased, the plasma level of ISDN was prolonged, but with no redn. in bioavailability. The systemic bioavailability of ISDN following transmucosal delivery through the oral mucosa can be modulated by controlling the ratio of ISDN loading in the fast- and sustained-release layers (F/S) as well as ISDN content in sustained-release layer. The effect was demonstrated in both in vitro and in vivo studies, which attained.

Pastino GM, Sultatos LG, Flynn EJ. **Development and application of a physiologically based pharmacokinetic model for ethanol in the mouse**. Alcohol Alcohol 1996;31(4):365-74.
The purpose of the present study was to develop a physiologically based pharmacokinetic (PBPK) model in the mouse and to utilize it to evaluate the relative contribution, if any, of gastric alcohol dehydrogenase (ADH) to the bioavailability of ethanol. The PBPK model developed in Swiss Webster male mice accurately simulated blood and brain ethanol concentrations following an intraperitoneal administration of 0.82 and 3.2 g of ethanol/kg body weight. Application of the model illustrated that inclusion of gastric ADH into the model provided a less accurate fit to the experimental data, and therefore gastric ADH did not contribute to the overall disposition of an orally administered ethanol dose of 0.75 g/kg. Furthermore, the model also indicated that changes in percentage cardiac output to the liver had a minimal effect on the blood ethanol concentration (BEC) time curve. The results illustrate the validity of the PBPK model developed for ethanol and demonstrate that in the Swiss Webster male

mouse the bioavailability of ethanol is minimally affected, if at all, by metabolism by gastric ADH.

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

Poulin P, Krishnan K. **Molecular structure-based prediction of the partition coefficients of organic chemicals for physiological pharmacokinetic models**. Toxicol Methods 1996;6(3):117-37.

Quaroni A, Hochman J. **Development of intestinal cell culture models for drug transport and metabolism studies**. Adv Drug Delivery Rev 1996;22(1-2):3-52.
CBAC COPYRIGHT: CHEM ABS A review with 417 refs. Cell culture models offer many advantageous features for the anal. of drug transport and drug metab. From a basic research perspective, these systems offer the potential for manipulating the environment or cellular properties as a means to address mechanistic questions. From a drug discovery perspective, cell culture models can be used to expedite identification of compds. with favorable pharmacokinetic properties, and to evaluate structure-absorption/metab. relationships. In this review, we will use the intestinal epithelium as an example for discussing issues assocd. with the development of new cell culture models for evaluating drug metab. Specifically, we will discuss biol. properties of the intestinal epithelium and address biol. and practical consideration in the application of tumor cell lines, short-term primary cultures, stem-like cell cultures, and oncogene immortalized cells as approaches to establishing models for the intestinal epithelium.

Saltzman BE. **Assessment of health effects of fluctuating concentrations using simplified pharmacokinetic algorithms**. J Air Waste Manag Assoc 1996;46(11):1022-34.

The purpose of this study was to develop a simple, practical method to improve the accuracy of assessing health effects by determining the concentration patterns inside the body resulting from fluctuating external pollutant concentrations. Linear pharmacokinetic processes were assumed, and the attenuations of the high- and low-frequency components of the external pattern when entering through the biological window were determined. Similar attenuations also were determined for the time-averaged sampling window. It was shown that when the averaging time was less than 1/4 of the biological half-life, no information of biological significance was lost. Thus, a simple arithmetic step equation was proposed to convert external time-averaged concentrations to biologically effective concentrations proportional to internal concentrations. Calculations could readily be made in real time by a monitoring instrument, or even with a pocket calculator. Another simple algorithm was proposed for determining a biological damage parameter representative of cumulative damage if the body repair process is slow. Finally, simple algorithms were proposed for calculating body burdens from total absorbed mass rates, a procedure that should be useful for pollutants such as lead that may enter the body through multiple pathways. Results were compared with experimental and hypothetical data to show their utility.

Schilter B, Perrin I, Cavin C, Huggett AC. **Placental glutathione S-transferase (GST-P) induction as a potential mechanism for the anti-carcinogenic effect of the coffee-specific components cafestol and kahweol**. Carcinogenesis 1996;17(11):2377-84.
The coffee specific diterpenes cafestol and kahweol (C + K) have been reported to be anti-carcinogenic in several animal models. It has been postulated that this activity may be related to their ability to induce glutathione S-transferases (GSTs). We investigated the influence of a mixture of C + K, incorporated at various levels in the diet of Sprague-Dawley rats, on the expression of different hepatic GST iso-enzymes. Liver samples were examined using isoform-specific GST substrates and antibodies, and highly selective oligomers were employed to determine effects at the RNA level. A dose-dependent increase in general GST activity was observed in male and female animals following 28 or 90 days of treatment. A time-course study demonstrated that the maximal effect was observed within 5 days of treatment. Little or no effect was found on the activity of GST alpha and mu iso-enzymes. The most striking observation was a dose-dependent induction of placental glutathione S-transferase (GST-P) which could be demonstrated at the mRNA, protein and enzymatic levels. This effect was observed in both male and female rats. The maximal induction was attained within 5 days of treatment with C + K, remained elevated with continued treatment, but was reversible on withdrawal of treatment. Immunohistochemical examination of liver slices revealed a strong even distribution of GST-P expression throughout the acinus at the highest dose of C + K, while at lower doses the induction of GST-P occurred predominantly in periportal hepatocytes. There was no indication of the presence of preneoplastic foci and, furthermore, the effect of C + K on the GST-P was completely reversible. These findings indicate that the anticarcinogenic mechanism of C + K may involve a specific induction of GST-P and suggest a potential role for GST-P in detoxifying carcinogenic compounds.

Scully P, Meehan E, Kelly JG. **High-performance liquid chromatographic assay for diltiazem in small-volume blood specimens and application to pharmacokinetic studies in rats**. J Chromatogr A 1996;729(1-2):297-300.
A high-performance liquid chromatographic (HPLC) method was developed which involves the use of

two 5-microns BDS silica gel columns (15 cm x 4.6 mm I.D.) in series for increased resolution and sensitivity, and an organic mobile phase for both extraction and elution of diltiazem. Plasma samples (400 microliters) were extracted using the organic mobile phase [n-hexane-methanol-dichloromethane-ammonia (370:35:30:0.3)] and the extracts were monitored at 240 nm. Desipramine (30 micrograms ml-1) was the internal standard. The limit of quantification in plasma was 20 ng ml-1 with a correlation coefficient of > or = 0.999 within the 20-800 ng ml-1 standard window. The inter- and intra-assay R.S.D. s were within 5%. The recovery of diltiazem varied from 101.1% at 20 ng ml-1 to 93.7% at 400 ng ml-1. The method was applied to the investigation of diltiazem absorption in a rat. Drug absorption was based on the intestinal single-pass perfusion model. The concentration of diltiazem in all test perfusion solutions was 1 mg ml-1 (2.4 mM) and the flow-rate through the system was 3.33.10(-3) ml s-1. A non-specific mucolytic absorption enhancer was also added to a diltiazem solution and studied in the in situ system. The pharmacokinetics of diltiazem hydrochloride were investigated in two study groups of Wistar rats (n = 4). A two-sample Student's t-test was employed to compare values of the area under the curve (AUC). The pharmacokinetic data indicated that the AUC in the group which received the enhancer [18.12 +/- 5.43 ng ml-1 h-1 (+/- S.D.)] was higher than that in the control group (11.49 +/- 3.67 ng h-1.

Semino G, Lilly P, Andersen ME. **A pharmacokinetic model describing pulsatile uptake of orally-administered carbon tetrachloride**. Toxicology 1997;117(1):25-33.
CBAC COPYRIGHT: CHEM ABS In this paper, we present a multi-compartmental description of the gastrointestinal (GI) tract linked to a physiol. based pharmacokinetic (PB-PK) model to describe the complex oral uptake of carbon tetrachloride (CCl4) administered in corn oil and 0.25% Emulphor. The GI submodel was described using a series of subcompartments, each subcompartment described with an absorption const. (Ka, 1/h), a bioavailability term (A, unitless), and a compartment emptying time (T, h). The model was parameterized by fitting multi-peak blood and exhaled breath chamber concn.-time profiles following oral gavage of CCl4 in corn oil and aq. vehicles to male Fischer 344 rats. Successful fitting of exptl. data was accomplished by varying values of Ka, A, and T until adequate fits were obtained. Values of Ka and A required to fit data from aq. gavage were greater than corn oil. Utilization of the multi-compartmental GI tract submodel provided increased precision in fitting complex oral uptake profiles compared to previously used one- and two-compartment oral uptake models. This model provides ests. of absorption rate consts. and bioavailabilities as well as providing a framework for generation of more complete, physiol.-realistic descriptions of oral absorption.

Shin J, Yoon Y, Cha I, Jang I, Lee K, Shin S. **[Pharmacokinetic modeling of reversible interconversion between prednisolone and prednisone]**. Taehan Yakrihak Chapchi 1996;32(2):269-81 . (Kor)
CBAC COPYRIGHT: CHEM ABS Pharmacokinetics of prednisolone and prednisone undergoing reversible interconversion were analyzed from the model including this metabolic process. Blood samples were drawn serially up to 12 h after I.V. bolus injection of 1 mg/kg prednisolone sodium phosphate and prednisone into 8 dogs as a crossover manner. Plasma concns. of those two steroids were simultaneously measured with the method of HPLC. After injection, plasma concns. of administered prednisolone and prednisone were declined with a biexponential pattern and their metabolic partner was rapidly formed. Plasma concns. of those metabolite were decayed in parallel with their parent steroids

through the elimination phase. Apparent clearances of prednisolone and prednisone were 11.12.0 mL/min/kg and 45.96.4 mL/min/kg, and they were underestimated by 29.4% and 33.6% compared to their real clearances(15.74.4 and 69.217.7 mL/min/kg) estd. using reversible interconversion model. Apparent vol. of distribution of prednisolone(1.32.+-.0.43 L/kg) and prednisone(4.81.+-.2.75 L/kg) were overestimated by 53.5 and 52.7% and were compared to the real vols. (0.86.+-.0.30 and 3.15.+-.2.13 L/kg). Mean residence time of prednisolone(2.0.+-.0.61 h) and prednisone(1.74.+-.0.74 h) were much longer than the real sojourn time(0.93.+-.0.26 and 0.88.+-.0.54 h). Essential clearances in the reversible interconversion were greater as following orders: Cl21(44.3 mL/min/kg) > Cl20(24.2 mL/min/kg) > Cl12 (7.9 mL/min/kg) > Cl10(7.8 mL/min/kg). Estd. mean values of RF, EE, %X1ss and RHO21 were 0.31. +-.0.10, 1.49.+-.0.23, 69.3.+-.16.7 % and 0.65.+-.0.10, resp. These results suggested that true pharmacokinetic parameters estd. from the model including reversible interconversion were significantly different from the apparent parameters estd. from the conventional mammillary model, and disposition of these two steroids seemed to be well explained by the model including reversible interconversion.

Shou M, Korzekwa KR, Krausz KW, Buters JT, Grogan J, Goldfarb I, Hardwick JP, Gonzalez FJ, Gelboin HV. **Specificity of cDNA-expressed human and rodent cytochrome P450s in the oxidative metabolism of the potent carcinogen 7,12-dimethylbenz[a]anthracene**. Mol Carcinog 1996;17 (4):241-9.
7,12-Dimethylbenz[a]anthracene (DMBA), a potent carcinogen, requires metabolic activation by cytochrome P450s (P450s) to electrophilic metabolites that result in DNA modification, mutagenicity, and carcinogenicity. In this study, we used eight human forms, four rodent forms, and one rabbit form of P450 expressed from recombinant vaccinia or baculovirus vectors to define their specificity for metabolizing DMBA. Of the eight human P450s, 1A1 was the most active (specific activity = 14.7 nmol/min/nmol of P450) in total metabolism of DMBA and showed approximately 6- to 33-fold more activity than other P450s, 2B6, 2C9, and 1A2 were also capable of metabolizing DMBA (2.0-2.5 nmol/min/nmol of P450), whereas 2C8, 2E1, 3A4, and 3A5 exhibited relatively low activities. Among animal P450s, mouse 1A1 exhibited activity similar to that of human 1A1 and had 5.0- to 37-fold more activity than other rodent and rabbit P450s. In regard to enzyme regioselectivity, most human and rodent P450s predominantly formed the 8,9-diol, but human 2B6 and rat 2B1 preferentially formed the 5,6-diol. In the production of monohydroxymethyl metabolites, all the enzymes yielded more 7-hydroxymethyl-12-methylbenz[a]anthracene (7HOM12MBA) than 12-hydroxymethyl-7-methylbenz[a]anthracene (7M12HOMBA), except for human 1A1, which presented the reverse selectivity. Human liver microsomes from 10 organ donors were shown to metabolize DMBA and in most circumstances generated the metabolic profile DMBA trans-8,9-dihydrodiol > 7HOM12MBA > or = DMBA trans-5,6-dihydrodiol > or = 7,12-dihydroxymethylbenz[a]anthracene > 7M12HOMBA > DMBA trans-3,4-dihydrodiol. Thus, the combined activity of hepatic microsomal 2C9, 1A2, and 2B6 may contribute to the metabolic activation and the metabolism of DMBA in normal human liver.

Shou M, Krausz KW, Gonzalez FJ, Gelboin HV. **Metabolic activation of the potent carcinogen dibenzo(a,h)anthracene by cDNA-expressed human cytochromes P450**. Arch Biochem Biophys 1996;328(1):201-7.
The metabolism of dibenzo(a,h)anthracene (53703) (DBA) by 3-methylcholanthrene induced rat and normal human liver microsomes was studied. Rat liver microsomes were harvested from Sprague-

Dawley-rats after treatment with four daily intraperitoneal injections of 25mg/kg 3MC. After incubation with DBA and NADPH for 30 minutes, the metabolites were isolated and characterized by high performance liquid chromatography and mass spectroscopy. Three dihydrodiol, one diphenol and three phenolic metabolites were identified. Recombinant human P450 proteins were incubated for 30 minutes (min) with radiotagged DBA and NADPH. The most active enzymes were 1A2 and 2C9. Moderate activity was seen for 2B6, and lower activities were seen for 2C8, 2E1, 3A3, 3A4 and 3A5. The 3,4-dihydrodiol was preferentially formed by 2C9, at a rate twofold higher than seen for 1A2 and 2B6, and 12 to 37 times the rate of the other P450s. Analysis of the regioselectivity of the enzymes showed that 1A2, 2B6 and 2C9 poorly oxidized the 5,6-position. While 1A2 was most active at the 1,2-position, 2B6 and 2C9 showed greatest activity at the 3,4-position of DBA. Of these three enzymes, 2C9 had the highest catalytic efficiency for formation of the 3,4-dihydrodiol. Metabolites of DBA by normal human liver microsomes from 14 individuals were formed at rates from 291 to 1,104pmol/min/nmolP450. The rates for 1,2-diol formation were highest followed by the rates for 3,4-diol, diphenol, total phenols and 5,6-diol. Overall, the pattern of DB(a,h)A metabolism by human liver microsomes was similar to that of the 1A2 enzyme. The authors conclude that 2C9, 1A2 and 2B6 are the major enzymes involved in the metabolic activation of DBA in the liver.

Shou M, Krausz KW, Gonzalez FJ, Gelboin HV. **Metabolic activation of the potent carcinogen dibenzo[a,l]pyrene by human recombinant cytochromes P450, lung and liver microsomes**. Carcinogenesis 1996;17(11):2429-33.
The metabolic activation of dibenzo[a,l]pyrene (DB[a,l]P), recently considered the most potent carcinogen among all polycyclic aromatic hydrocarbons, to the 11,12-dihydrodiol, a precursor of the ultimate carcinogens, the 11,12-diol-13,14-epoxides, was investigated using eleven human recombinant cytochrome P450s, as well as human lung and liver microsomes. Of all human P450s, 1A1 was the most active in the metabolism of DB[a,l]P (310 pmol/min, nmol P450) and had 5-23-fold higher catalytic activity than other P450s examined. The order of activity in the formation of the 11,12-dihydrodiol was as follows: 1A1 (116 pmol/min, nmol P450) > 2C9 (29) > 1A2 (22) > 2B6 (18) > 3A4 (16) > others (< or = 5). The Km of 1A1 for DB[a,l]P and Vmax for the formation of 11,12-dihydrodiol were 3.9 microM and 0.13/min, respectively. Liver microsomes from 14 individuals were shown to metabolize DB[a,l]P and the rates for production of 11,12-dihydrodiol ranged from 4 to 71 pmol/min.

Singh BR, Li B, Read D. **Botulinum versus tetanus neurotoxins: why is botulinum neurotoxin but not tetanus neurotoxin a food poison?** Toxicon 1995;33(12):1541-7.
Botulinum and tetanus neurotoxins, produced by Clostridium botulinum and Clostridium tetani, respectively, are the most poisonous poisons known to mankind. Although botulinum and tetanus neurotoxins share several characteristics, such as similar mol. wts, similar macrostructure, virtually identical mode of action, and a strong amino acid sequence homology, the two neurotoxins differ in one very significant way; only botulinum neurotoxin is a food poison. Factors responsible for the food poisoning potential of botulinum neurotoxins seem to be a group of complexing proteins that are also produced by C. botulinum, and are known to associate with the neurotoxin. Translation products of nucleotide sequences upstream to the neurotoxin genes of serotypes A, B, C, D, E and F botulinum neurotoxin reveal the location of genes for one of the complexing proteins that could be transcribed as polycistronic mRNA to include neurotoxin sequences. No such protein seems to be present in C. tetani,

suggesting that the lack of complexing proteins might be responsible for tetanus not being a food poison.

Sweeney LM, Himmelstein MW, Schlosser PM, Medinsky MA. **Physiologically based pharmacokinetic modeling of blood and tissue epoxide measurements for butadiene**. Toxicology 1996;113(1-3):318-21.
In vitro and in vivo butadiene (BD) metabolism data from laboratory animals were integrated into a rodent physiologically based pharmacokinetic (PBPK) model with flow- and diffusion-limited compartments. The resulting model describes experimental data from multiple sources under scenarios such as closed chamber inhalation and nose-only flow-through inhalation exposures. Incorporation of diurnal glutathione (GSH) variation allows accurate simulation of GSH changes observed in air control nose-only exposures and BD exposures. An isolated tissue model based on rate parameters determined in vitro predicts the decrease in epoxide concentrations in intact animals during the time lag between exsanguination and tissue removal for tissues capable of epoxide biotransformation, providing a better indication of in vivo dosimetry. Further refinements of the model are required relative to model predictions of an important BD metabolite, diepoxybutane.

Thomas RS, Bigelow PL, Keefe TJ, Yang RS. **Variability in biological exposure indices using physiologically based pharmacokinetic modeling and Monte Carlo simulation**. Am Ind Hyg Assoc J 1996;57(1):23-32.
A physiologically based pharmacokinetic model coupled with Monte Carlo simulation was used to estimate worker exposure to benzene (71432), carbon-tetrachloride (56235), chloroform (67663), methyl-chloroform (71556), methyl-chloride (74873), and trichloroethylene (79016), and these results were compared with current biological exposure indices (BEI). The model indicated that, for all six solvents, expired air concentrations would be suitable biological indices if the type of expired air was specified. The current BEI for benzene in end exhaled air was estimated by the model to protect 95% of the worker population. The current BEI for methyl-chloroform, however, was found to be much lower than corresponding model predictions, protecting less than 10% of the worker population. The model also suggested that the current BEI for phenol (108952) following benzene exposure would protect 68% of the worker population. Fifty four percent and 97% of the worker population would be protected using estimates for the methyl-chloroform determinants trichloroacetic-acid (76039) and trichloroethanol (115208), respectively; when these were used as determinants of trichloroethylene exposure, 84% of the workers were estimated to be protected by the current BEI. The authors conclude that current BEIs may not be consistently protecting workers.

Thomas RS, Yang RS, Morgan DG, Moorman MP, Kermani HR, Sloane RA, O'Connor RW, Adkins B Jr, Gargas ML, Andersen ME. **PBPK modeling/Monte Carlo simulation of methylene chloride kinetic changes in mice in relation to age and acute, subchronic, and chronic inhalation exposure**. Environ Health Perspect 1996;104(8):858-65.
During a 2-year chronic inhalation study on methylene chloride (2000 or 0 ppm; 6 hr/day, 5 days/week), gas-uptake pharmacokinetic studies and tissue partition coefficient determinations were conducted on female B6C3F1, mice after 1 day, 1 month, 1 year, and 2 years of exposure. Using physiologically based pharmacokinetic (PBPK) modeling coupled with Monte Carlo simulation and bootstrap resampling for data analyses, a significant induction in the mixed function oxidase (MFO) rate const. (Vmaxc) was obsd. at the 1-day and 1-mo exposure points when compared to concurrent control mice, while decreases

in glutathione S-transferase (GST) rate const. (Kfc) were obsd. in the 1-day and 1-mo exposed mice. Within exposure groups, the parent Vmaxc maintained significant increases in the 1-mo and 2-yr control groups. Although the same initial increase exists in the exposed group, the 2-yr Vmaxc is significantly smaller than the 1-mo group ($p < 0.001$). Within-group differences in median Kfc values show a significant decrease in both 1-mo and 2-yr groups among control and exposed mice ($p < 0.001$). Although no changes in methylene chloride soly. as a result of prior exposure were obsd. in blood, muscle, liver, or lung, a marginal decrease in the far:air partition coeff. was found in the exposed mice at $p = 0.053$. Age related soly. differences were found in muscle:air, liver:air, lung:air, and fat:air partition coeffs. at $p < 0.001$, while the soly. of methylene chloride in blood was not affected by age ($p = 0.461$). As a result of this study, the authors conclude that age and prior exposure to methylene chloride can produce notable changes in disposition and metab. and may represent important factors in the interpretation of toxicol. data and its application to risk assessment.

Troconiz IF, Garrido MJ, Garcia E, Suarez E, Calvo R. **Pharmacokinetic-pharmacodynamic modeling of mivacurium in rats**. J Pharm Sci 1997;86(2):252-6.
CBAC COPYRIGHT: CHEM ABS The pharmacokinetic (PK) and pharmacodynamic (PD) characteristics of the neuromuscular blocking agent mivacurium were evaluated sep. in two groups of rats receiving 0.6 mg kg-1 of mivacurium in a 2.5-min i.v. continuous (i.v.) infusion. The PK parameters for mivacurium were detd. in the first group. A two-compartment model describes the kinetics of mivacurium in plasma. The ests. of the apparent vol. of distribution at steady-state and plasma clearance [mean(SE)] were 650 (123) mL kg-1 and 9.9 (0.75) mL min-1 kg-1, resp. In the second group, the evoked tibialis anterior muscle tension was monitored. The PK parameters derived from the first group were used to compute mivacurium plasma concns. (C) at the times the PD measurements were recorded in the second group. The concn.-neuromuscular effect [% depression of initial twitch tension (E)] relationship was analyzed by two approaches. (1) The relationship of estd. effect site concns. vs. E; a sigmoidal Emax model described the effect compartment concns. vs. E relationship. The est. [mean(SE)] of Cess50 (steady-state plasma concn. eliciting half of max. E) was 0.65 (0.01) mug mL-1. The value [mean(SE)] of keo (rate const. of equilibration between plasma and effect site) was estd. at 0.32 (0.03) min-1. (2) The relationship of descending limb C vs. E; a sigmoidal Emax model described such relationship. The est. [mean(SE)] of C50 (post-infusion C eliciting half of max. E) was 0.57(0.03) mug mL-1. The PD properties of mivacurium were also evaluated in another two groups of animals receiving either 5- or 10-min continuous i.v. infusion; PK and PD parameters obtained from the 2.5-min infusion expts. were used to predict the time course of E in the groups receiving 0.6 mg kg-1 of mivacurium in 5- and 10-min infusions; simulations using the estd. parameters.

Turesky RJ, Markovic J, Aeschlimann JM. **Formation and differential removal of C-8 and N2-guanine adducts of the food carcinogen 2-amino-3-methylimidazo[4,5-f]quinoline in the liver, kidney, and colorectum of the rat**. Chem Res Toxicol 1996;9(2):397-402.
Chronic feeding of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) in the diet results in tumor formation of the liver and colorectum, but does not induce tumorigenesis in the kidney of male Fischer-344 rats. The formation and rate of removal of DNA adducts were investigated in rats given an oral dose of IQ (20 mg/kg) to determine if adduct persistence affects the tissue susceptibility to IQ-induced tumorigenesis. Analysis of DNA adducts by 32P-postlabeling showed the formation of two 2'-

deoxyguanosine (dG) adducts, N-(deoxyguanosin-8-yl)-2-amino-3-methylimidazo[4,5-f]quinoline (dG-C8-IQ) and 5-(deoxyguanosin-N2-yl)-amino-3-methylimidazo[4,5-f]quinoline (dG-N2-IQ) The pattern and distribution of these dG adducts were similar in all tissues; dG-C8-IQ and dG-N2-IQ accounted for approximately 70% and 15-20%, respectively, of the observed radioactivity. Maximal DNA binding was observed in liver (7.64 +/- 1.08 adducts per 10(7) bases) and in colorectum (1.08 +/- 0.22 adducts per 10 (7) bases) 24 h following IQ treatment, while optimal binding appeared in kidney (2.41 +/- 0.47 adducts per 10(7) bases) 72 h after treatment. Greater than 50% of the dG-C8-IQ adduct was removed from DNA of liver and kidney within 1 week of treatment. In contrast, the dG-N2-IQ adduct persisted and was the principal lesion remaining in liver and kidney 4 weeks after treatment with IQ. There was no evidence for selective removal of either adduct in the colorectum over a 3 week period, and adduct removal appeared to be attributed to cell turnover and not due to excision repair processes. Therefore, the relative persistence of dG-C8-IQ and dG-N2-IQ adducts doses not appear to explain tissue susceptibility to IQ-induced neoplasia. The slow disappearance of IQ-DNA adducts suggests that adducts may accumulate during chronic exposure to IQ. Further investigations on DNA adduct formation and removal in animals chronically exposed to this carcinogen may help to explain the susceptibility of various organs to IQ-induced tumorigenesis.

Ueng TH, Kang JJ, Chao IC, Chen YC. **[Cytochrome P450: enzyme regulation and toxicological significance]**. J Food Drug Anal 1996;4(1):13-23. (Chi)
BIOSIS COPYRIGHT: BIOL ABS. The pharmacological and toxicological responses of drugs and environmental chemicals are dependent upon metabolism of the compounds. Cytochrome P450 (P450)-dependent monooxygenases are the primary enzyme system responsible for the oxidative metabolism of drugs, carcinogens, food additives, and environmental chemicals. The underlying basis for the broad substrate specificity of P450 is that there are multiple forms of P450. A nomenclature system based on amino acid sequence homology is adopted for the increasing number of P450 hemoproteins. P450 genes are subject to regulation by many physiological and environmental factors. Regulation of P450 enzymes shows marked species and tissue specificity which plays an important role in species and tissue extrapolation of chemical risk assessment and target organ toxicity. P450 isoforms are readily inducible following exposure to foreign compounds of which phenobarbital, 3-methylcholanthrene, and ethanol are the prototypic inducing agents. Many drugs and natural compounds are potent inhibitors of P450 and dependent catalytic activity. Mechanism of P450 regulation also shows multiplicity at the transcriptional, translational, and post-translational stages. Polymorphism and racial difference are important determinants of drug metabolism and susceptibility to diseases in humans. Traditional Chinese medicines have the ability to induce and inhibit multiple forms of P450 monooxygenases. Expression of human P450 in mammalian cell lines provides an alternative system to study the functions of P450s in xenobiotic metabolism and toxicity in humans. P450 may be associated with regulation of cellular entry of calcium. Finally, the perspective of future P450 research is discussed.

Urbanska EM, Drelewska E, Borowicz KK, Blaszczak P, Kleinrok Z, Czuczwar SJ. **NG-nitro-L-arginine, a nitric oxide synthase inhibitor, and seizure susceptibility in four seizure models in mice**. J Neural Transm 1996;103(10):1145-52.
CBAC COPYRIGHT: CHEM ABS Nitric oxide may be involved in seizure phenomena even though data often seem to be contradictory. This prompted us to study the influence of nitric oxide upon elec.

and chem. induced seizures. The effects of nitric oxide synthase inhibitor, NG-nitro-L-arginine (NNA), on pentylenetetrazol-, aminooxyacetic acid-, aminophylline-induced seizures or electroconvulsive shock were evaluated. NNA was applied at 1, 10 and 40mg/kg 0.5 and 2.0h before chem. seizures and at 1 and 40mg/kg 0.5 and 2.0h prior to electroconvulsions. The nitric oxide synthase inhibitor (up to 40mg/kg) did not affect the susceptibility of mice to pentylenetetrazol, amino-oxyacetic acid or electroconvulsions. However, NNA significantly enhanced the convulsive properties of aminophylline when applied at 40mg/kg, 0.5h before the test. The CD50 value for aminophylline-induced clonus and tonus/mortality was decreased from 233 to 191 and from 242 to 212mg/kg, resp. However, this pretreatment also led to a significant increase in the plasma levels of theophylline. Our results suggest that differential effects of NNA on chem.-induced convulsions might in some cases be assocd. with a pharmacokinetic interaction.

Verna L, Whysner J, Williams GM. **2-acetylaminofluorene mechanistic data and risk assessment dna reactivity enhanced cell proliferation and tumor initiation**. Pharmacol Ther 1996;71(1-2):83-105.
BIOSIS COPYRIGHT: BIOL ABS. RRM LITERATURE REVIEW MOUSE 2-ACETYLAMINOFLUORENE DNA REACTIVITY BLADDER CARCINOGENESIS MATHEMATICAL DATA ANALYTICAL METHOD.

Verna L, Whysner J, Williams GM. **N-nitrosodiethylamine mechanistic data and risk assessment bioactivation dna-adduct formation mutagenicity and tumor initiation**. Pharmacol Ther 1996;71(1-2):57-81.
BIOSIS COPYRIGHT: BIOL ABS. RRM literature review rat n-nitrosodiethylamine mutagenicity carcinogenesis liver mathematical data analytical method.

Volkmer H, Leuschner R, Zacharias U, Rathjen FG. **Neurofascin induces neurites by heterophilic interactions with axonal NrCAM while NrCAM requires F11 on the axonal surface to extend neurites**. J Cell Biol 1996;135(4):1059-69.
Neurofascin and NrCAM are two axon-associated transmembrane glycoproteins belonging to the L1 subgroup of the Ig superfamily.

Von Moltke LL, Greenblatt DJ, Schmider J, Duan SX, Wright CE, Harmatz JS, Shader RI. **Midazolam hydroxylation by human liver microsomes in vitro: inhibition by fluoxetine, norfluoxetine, and by azole antifungal agents**. J Clin Pharmacol 1996;36(9):783-91.
Biotransformation of the imidazobenzodiazepine midazolam to its alpha-hydroxy and 4-hydroxy metabolites was studied in vitro using human liver microsomal preparations. Formation of alpha-hydroxy-midazolam was a high-affinity (Km = 3.3 mumol/L) Michaelis-Menten process coupled with substrate inhibition at high concentrations of midazolam. Formation of 4-hydroxy-midazolam had much lower apparent affinity (57 mumol/L), with minimal evidence of substrate inhibition. Based on comparison of Vmax/Km ratios for the two pathways, alpha-hydroxy-midazolam formation was estimated to account for 95% of net intrinsic clearance. Three azole antifungal agents were inhibitors of midazolam metabolism in vitro, with inhibition being largely consistent with a competitive mechanism. Mean competitive inhibition constants (Ki) versus alpha-hydroxy-midazolam formation were 0.0037 mumol/L for ketoconazole, 0.27 mumol/L for itraconazole, and 1.27 mumol/L for fluconazole. An in vitro-in vivo scaling model predicted inhibition of oral midazolam clearance due to coadministration of

ketoconazole or itraconazole; the predicted inhibition was consistent with observed interactions in clinical pharmacokinetic studies. The selective serotonin reuptake inhibitor (SSRI) antidepressant fluoxetine and its principal metabolite, norfluoxetine, also were inhibitors of both pathways of midazolam.

Watanabe KH, Bois FY. **Interspecies extrapolation of physiological pharmacokinetic parameter distributions**. Risk Anal 1996;16(6):741-54.
Three methods (multiplicative, additive, and allometric) were developed to extrapolate physiological model parameter distributions across species, specifically from rats to humans. In the multiplicative approach, the rat model parameters are multiplied by the ratio of the mean values between humans and rats. Additive scaling of the distributions is defined by adding the difference between the average human value and the average rat value to each rat value. Finally, allometric scaling relies on established extrapolation relationships using power functions of body weight. A physiologically-based pharmacokinetic model was fitted independently to rat and human benzene disposition data. Human model parameters obtained by extrapolation and by fitting were used to predict the total bone marrow exposure to benzene and the quantity of metabolites produced in bone marrow. We found that extrapolations poorly predict the human data relative to the human model. In addition, the prediction performance depends largely on the quantity of interest. The extrapolated models underpredict bone marrow exposure to benzene relative to the human model. Yet, predictions of the quantity of metabolite produced in bone marrow are closer to the human model predictions. These results indicate that the multiplicative and allometric techniques were able to extrapolate the model parameter distributions, but also that rats do not provide a good kinetic model of benzene disposition in humans.

Whysner J, Conaway CC, Verna L, Williams GM. **Vinyl chloride mechanistic and risk assessment DNA reactivity and cross-species quantitative risk extrapolation**. Pharmacol Ther 1996;71(1-2):7-28.
BIOSIS COPYRIGHT: BIOL ABS. RRM Literature review human rodent vinyl chloride mutagenesis angiosarcoma carcinogenesis occupational exposure mathematical data analytical method.

Whysner J, Ross PM, Williams GM. **Phenobarbital mechanistic data and risk assessment enzyme induction enhanced cell proliferation and tumor promotion**. Pharmacol Ther 1996;71(1-2):153-91.
BIOSIS COPYRIGHT: BIOL ABS. RRM Literature review mouse rat phenobarbital enzyme induction liver tumor carcinogenesis mathematical data analytical method.

Whysner J, Verna L, Williams GM. **Benzidine mechanistic data and risk assessment species-and organ-specific metabolic activation**. Pharmacol Ther 1996;71(1-2):107-26.
BIOSIS COPYRIGHT: BIOL ABS. RRM literature review mouse rat hamster rabbit dog human benzidine organ-specific metabolic activation carcinogenesis liver mathematical data analytical method.

Whysner J, Williams GM. **2 3 7 8-tetrachlorodibenz o-p-dioxin mechanistic data and risk assessment gene regulation cytotoxicity enhanced cell proliferation and tumor promotion** .
Pharmacol Ther 1996; 71(1-2):193-223.
BIOSIS COPYRIGHT: BIOL ABS. RRM Literature review human rodent 2 3 7 8-tetrachlorodibenzo-p-dioxin toxicity biodistribution carcinogenesis mathematical data analytical method.

Whysner J, Williams GM. **Butylated hydroxyanisole mechanistic data and risk assessment conditional species-specific cytotoxicity enhanced cell proliferation and tumor promotion**. Pharmacol Ther 1996;71(1-2):137-51.
BIOSIS COPYRIGHT: BIOL ABS. RRM Literature review human rodent.

Whysner J, Williams GM. **D-limonene mechanisitic data and risk assessment absolute species-specific cytotoxicity enhanced cell proliferation and tumor promotion**. Pharmacol Ther 1996;71(1-2):127-36. BIOSIS COPYRIGHT: BIOL ABS. RRM Literature review human rat d-limonene enhanced cell proliferation toxicity carcinogenesis mathematical data analytical method.

Whysner J, Williams GM. **Saccharin mechanistic data and risk assessment urine composition enhanced cell proliferation and tumor promotion**. Pharmacol Ther 1996;71(1-2):225-52.
BIOSIS COPYRIGHT: BIOL ABS. RRM literature review human rat sodium saccharin male bladder tumor life time exposure carcinogenesis mathematical data analytical method.

Witcher JW, Boudinot FD. **Applications and simulations of a discontinuous oral absorption pharmacokinetic model**. Pharm Res 1996;13(11):1720-4.
CBAC COPYRIGHT: CHEM ABS To illustrate the application of a discontinuous oral absorption model to cimetidine and ranitidine plasma concn. vs. time data to demonstrate the use of the model for drugs which display discontinuous oral absorption profiles, and to illustrate the effect of various model parameters on plasma drug concn. vs. time profiles and bioavailability. A discontinuous oral absorption model was used to fit ranitidine and cimetidine serum concns. following oral and i.v. administration. The model was also used to simulate bioavailability and plasma concns. vs. time profiles for various parameter values. Serum concns. following administration of ranitidine and cimetidine were well described by the model, and parameter ests. obtained were in agreement with literature values. Simulations demonstrate the effects of various absorption parameters and gastrointestinal tract transit parameters on bioavailability and plasma concn. profiles.

Wu G. **Fit fluctuating blood drug concentration: a beginner's first note**. Pharmacol Res 1996; 33 (6):379-83.
CBAC COPYRIGHT: CHEM ABS In linear pharmacokinetics a blood drug concn. can be described and fitted using the equation of a sum of exponential functions, but this equation cannot exactly describe and fit a fluctuating (oscillating) blood drug concn., which is a common phenomenon in clin. pharmacokinetic settings. Although a no. of hypotheses have been provided for the phenomenon of a fluctuating blood drug concn., a possible math anal. has yet to be fully explored. In the present study we proposed several equations from other research fields to be used in pharmacokinetics. These equations contain decaying exponential sinusoidal functions and can theor. fit a fluctuating curve. We used these equations to produce fluctuating curves. The results suggested that these equations might be suitable for use in pharmacokinetics. Furthermore, we demonstrated that the anal. soln. of the differential equation system for a compartmental model can contain decaying exponential sinusoidal functions. We conclude that the anal. soln. of the differential equation system for a compartmental model in pharmacokinetics is a sum of decaying exponential and sinusoidal functions rather than a sum of exponential functions, the latter one being only an approx. anal. soln. of the differential equation system for a compartmental model.

Yamaguchi R, Hirano T, Asami S, Chung MH, Sugita A, Kasai H. **Increased 8-hydroxyguanine levels in DNA and its repair activity in rat kidney after administration of a renal carcinogen, ferric nitrilotriacetate**. Carcinogenesis 1996;17(11):2419-22.
The renal carcinogen, ferric nitrilotriacetate (Fe-NTA), is known to induce oxidative stress and the subsequent formation of a type of oxidative DNA damage, 8-hydroxyguanine (8-OH-Gua), in the rat kidney (Umemura et al., 1990). Using an improved DNA isolation method (Nakae et al., 1995), which reduces the background level of 8-OH-Gua, we found a five-fold increase in the 8-OH-Gua level in kidney DNA after a single i.p. injection of Fe-NTA. On the basis of the report that 8-OH-Gua repair activity is enhanced after cells are exposed to oxidative stress due to ionizing radiation (Bases et al., 1992), the measurement of 8-OH-Gua repair activity will also be useful to assess cellular oxidative stress. The 8-OH-Gua repair enzyme activity was determined with an endonuclease assay using a 22 mer DNA that contains 8-OH-Gua at a specific position. A five-fold increase in the 8-OH-Gua repair activity as compared with the control, was observed in the target organ, the rat kidney, 120 h after Fe-NTA administration. In the non-target organ, the liver, the increase was not as large (two-fold). This simple assay of oxidative DNA damage repair will be useful for evaluating the carcinogenicity of oxygen radical forming chemicals, in addition to chemical analyses of oxidative DNA damage.

Yamaguchi T, Yabuki M, Saito S, Watanabe T, Nishimura H, Isobe N, Shono F, Matsuo M. **Research to develop a predicting system of mammalian subacute toxicity. 3. Construction of a predictive toxicokinetics model**. Chemosphere 1996;33(12):2441-68.
A new predictive toxicokinetics model was developed to estimate subacute toxicity (target organs, severity, etc.) of non-congeneric industrial chemicals, where the chemical structures and physico-chemical properties are only available. Thus, a physiological pharmacokinetics model, which consists of blood, liver, kidney (these were experimentally found as major toxicological targets), muscle and fat compartments, was established to simulate the chemical concentrations in organs/tissues with pharmacokinetic parameters by means of Runge-Kutta-Gill algorithm. The pharmacokinetic parameters, i.e. absorption rate, absorption ratio, hepatic extraction ratio of metabolism and renal clearance were calculated by using separately established Quantitative Structure-Pharmacokinetics Relationship equations. The developed predictive model was then applied to simulations of 43 non-congeneric industrial chemicals. The chemical concentrations in organs/tissues after single oral administration were simulated, and their maximum concentrations (Cmax's) and area under the concentration-time curves (AUC's) were calculated. Fast Inverse Laplace Transform was newly applied for the purpose of simulation of 28-day repeated dose toxicity. Simulated concentrations of 28 days repeated dose were, however, found to be the same as those of simple repetitions of a single administration per day because of the short half-lives of non-congeneric industrial chemicals. A comparison of subacute toxicity data with Cmax's and AUC's in a single dose scenario suggested that the organs/tissues with relatively high concentrations of tested chemical substances were the most sensitive targets within a chemical. Chemical concentrations in liver, for instance, were correlated with the severity of hepatotoxicity among the chemicals. It was also suggested that to improve and widen the present approach, data of metabolite and reactivity of non-congeneric industrial chemicals to organs/tissues, receptors, etc. should be incorporated into the model.

Zhen J, Qi G, Zhang L. **[Comparison of pharmacokinetic parameters of amikacin between artery**

and vein of health and scald rabbits]. Zhongguo Yaoxue Zazhi 1996;31(9):539-42 . (Chi)
CBAC COPYRIGHT: CHEM ABS The differences of pharmacokinetic parameters of amikacin in artery and vein of healthy and scald rabbits were studied. Amikacine 22.5 mg kg-1 was given i.v. to healthy and scald (scald area > 20%) rabbits and blood was collected from artery and vein at different time to det. the blood concn. by EPIA. The distributions of amikacin in health and scald rabbits conformed to the two-compartment open model. Vc and Cl had significant differences after statistical anal. The results suggest that when using amikacin, the blood amikacin concn. must be monitored in scald treatment

Zheng NX, Sato H, Adachi I, Horikoshi I. **Pharmacokinetic and pharmacodynamic modeling of a thromboxane synthetase inhibitor, ozagrel, in humans**. Byoin Yakugaku 1996;22(1):26-37.
IPA COPYRIGHT: ASHP Pharmacokinetic and pharmacodynamic (PK/PD) analyses for ozagrel were performed to predict its PK/PD profiles in humans; the pharmacodynamics of ozagrel were characterized by serum levels of thromboxane B2 (TXB2), a pharmacological marker for thromboxane synthetase inhibition. Based on a quantitative relationship between the plasma ozagrel and serum TXB2 concentrations after single oral dosage, IC50 and Emax values were estimated to be 11.8 ng/ml and 94%, respectively. Pharmacokinetic parameters of ozagrel were estimated from plasma ozagrel concentrations after intravenous (IV) bolus, IV infusion, and oral administration at various doses. An integrated simple PK/PD model was developed to simulate the changes in serum TXB2 levels after oral administration at various doses, and the obtained simulation curves corresponded well with observed data. The predictability of the PK/PD model was evaluated by using the model to predict changes in serum TXB2 levels after multiple oral administration in humans. The predicted curves were in good agreement with observed data.

PULMONARY TOXICITY

Law F Cp, He SX, Chui YC. **Biotransformation and elimination of 1-nitropyrene in the isolated perfused lung: Effects of pretreating rats with phenobarbitone, beta-naphthoflavone, benz(a) anthracene or their mixtures**. Yaoxue Xuebao 1996;31(8):568-76.
BIOSIS COPYRIGHT: BIOL ABS. The isolated perfused rat lung (IPL) was perfused with 60 ml of recirculating Krebs-Ringer solution containing 150 mug of 1-nitropyrene (1-NP) for 1 h. The 1-NP was administered to the IPL by the intratracheal or intravascular route. At specific time points after 1-NP administration, perfusate samples were removed from the IPL and analysed for 1-NP and its metabolites by HPLC. Monohydroxynitropyrenes, dihydroxynitropyrenes and 1-NP were found to be present in the perfusate. The time course of 1-NP concentrations in the perfusate could be described by one-compartment pharmacokinetic model. Pretreatment of rats with beta-naphthoflavone (BNF), benz(a) anthracene (BA) or a mixture of phenobarbitone (PB) and BNF (PB + BNF) significantly enhanced the metabolism of 1-NP and decreased the mean residence time (MRT) of 1-NP in the perfusate. Pretreatment of rats with these mixed-function oxidase inducers also increased significantly the absorption of 1-NP by the lung when it was administered intratracheally. In contrast, pretreatment of rats with PB did not appear to have any effect on the pharmacokinetics of 1-NP in the IPL.

Leclaire RD, Hunt RE, Bavari S, Estep JE, Nelson GO, Wilhelmsen CL. **Potentiation of inhaled staphylococcal enterotoxin B-induced toxicity by lipopolysaccharide in mice**. Toxicol Pathol 1996;24 (5):619-26.
BIOSIS COPYRIGHT: BIOL ABS. Nonhuman primates are the established model for evaluating toxic responses to staphylococcal enterotoxins (SEs), as they react similarly to humans. Rodents are generally considered unresponsive to SEs. Binding affinities and T-cell reactivity suggest that SE binds more efficiently to primate major histocompatability complex class II receptors than to mouse receptors. We investigated the potentiation of staphylococcal enterotoxin B (SEB) inhalation toxicity by lipopolysaccharide (LPS) in BALB/c mice. Lethality occurred only when SEB was potentiated by LPS. Neither SEB nor LPS produced lethal effects alone. Temporal responses of interleukin 1alpha, tumor necrosis factor alpha, interleukin 2, and interferon-gamma evoked by inhaled SEB were enhanced by LPS. By 24 hr after intoxication, serum cytokines decreased to baseline levels, and consistent pulmonary perivascular leukocytic infiltrates were evident histologically. Histologic lesions induced by inhalation exposure to SEB by mice, with without potentiation by LPS, were similar to those in the rhesus monkey. Predominant pulmonary lesions included severe, diffuse interstitial and alveolar pulmonary edema, leukocytic infiltrates, mild perivascular edema, and alveolar fibrin deposition. Although the mechanism of aerosolized SEB-induced toxicity has not been completely resolved, similarities in histologic lesions, cytokine responses, and acute dose-response suggest the LPS-potentiated mouse model may be a credible alternative to the nonhuman primate model.

Lee JG, Madden MC, Reed W, Adler K, Devlin R. **The use of the single cell gel electrophoresis assay in detecting DNA single strand breaks in lung cells in vitro**. Toxicol Appl Pharmacol 1996; 141 (1):195-204.
DNA single strand breaks (SSB) can be used as a biomarker of oxidant exposure, and also as an indicator of the carcinogenicity/ mutagenicity of a substance. The single cell gel electrophoresis (SCGE) assay is more sensitive and requires fewer cells compared to other techniques used for detecting SSB. We examined the utility of using the SCGE assay for human lung cells exposed to endogenous and exogenous oxidants. A human bronchial cell line (BEAS) was used as a model of airway epithelial cells in this study. BEAS cells exposed to 0-50 microM hydrogen peroxide (H2O2) for 60 min at 4 degrees C exhibited a concentration-dependent increase in SSB as determined by an increased DNA migration area in a gel undergoing electrophoresis. H2O2-induced increases in DNA SSB were also demonstrated using cultured normal human tracheobronchial epithelial (NHBE) cells and human alveolar macrophages in a concentration response manner. BEAS cells were also exposed to air or ozone (O3) on a Transwell filter without medium present apically. Cells exposed to O3 at 0.1 or 0.4 ppm at 37 degrees C for up 120 min had a time- and concentration-dependent increase in SSB compared to air-exposed cells. NHBE cells exposed to 0.4 ppm O3 (60 min) also had increased DNA SSB. Cells with H2O2-induced DNA SSB can be frozen and stored up to 4 weeks without altering the original DNA SSB. These findings indicate that SCGE can be used to detect SSB in cultured lung cells, and has applicability for detecting SSB in lung cells recovered from in vivo and in vitro exposures to oxidants.

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

BIOSIS COPYRIGHT: BIOL ABS. RRM RESEARCH ARTICLE HAMSTER RAT ETHYLNITROSOUREA CARCINOGEN DIETHYLNITROSAMINE O-ETHYL-DEOXYGUANOSINE CELL-TYPE SPECIFIC TUMOR FORMATION CELL-TYPE SPECIFIC DNA-ADDUCT FORMATION CELL-TYPE SPECIFIC DNA-ADDUCT ELIMINATION RESPIRATORY TISSUE TOXICOLOGY.

Zoller T, Brehm M, Zeller WJ. **Differentiated HL-60 cells as model system for toxicity studies of inhalable dusts**. Toxicol Lett 1996;89(2):107-13.
For the assessment of the toxicity of inhalable dusts, generally either in vivo investigations or tests with cultures of freshly isolated macrophages are used. In the present study, a calcitriol-treated HL-60 tumor cell line was investigated as an alternative model and compared with bovine alveolar macrophages. In both systems, upon exposure to two different quartz fractions, the amount and reaction pattern of superoxide anion release (O2.-) were determined by lucigenin-enhanced chemiluminescence; viability of cells was determined using the fluorescein diacetate-ethidium bromide assay. Nearly equal results were obtained in both systems, recommending the differentiated HL-60 cell line as an easily manageable model system for toxicity studies of particulate bronchopulmonary noxious agents, especially with regard to the lower experimental expenditure.

QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIPS

Augelli-Szafran CE, Horwell DC, Kneen C, Ortwine DF, Pritchard MC, Purchase TS, Roth BD, Trivedi BK, Hill D, et al. **Cholecystokinin B antagonists. Synthesis and quantitative structure-activity relationships of a series of C-terminal analogs of CI-988**. Bioorg Med Chem 1996;4(10):1733-45.
CBAC COPYRIGHT: CHEM ABS A study of structure-activity relationships of a series of dipeptoid CCK-B receptor antagonists was performed in which variations of the Ph ring were examd. while the [(2-adamantyloxy)carbonyl]-alpha-methyl-(R)-tryptophan moiety of the potent antagonist CI-988 was kept const. Since the main focus of this study was Ph substituent variation, series design techniques were employed to insure an adequate spread of physicochem. properties (lipophilic, steric, and electronic), as well as positional substitution. A QSAR anal. on sets of 26 and 16 analogs revealed that CCK-B affinity was related to a combination of the overall size and, marginally, lipophilicity of the Ph ring substituents (i.e., smaller groups were assocd. with increased potency with an optimum pi near zero). Further exploration revealed that the dimensions and electronics of the para-Ph substituent could be related to CCK-B affinity. Increased affinity was seen with short, bulky (branched) electron-withdrawing groups. Analogs with small para-substituents appeared to be .apprx.1000-fold CCK-B selective, indicating that selectivity for CCK-B binding is sensitive to Ph ring substitution. The 4-F-Ph dipeptoid, derived from this study, has extraordinarily high affinity at the CCK-B receptor (IC50 = 0.08 nM) and was also very selective (940-fold CCK-B selective). Consistent with previous reports, (S)-configuration at the substituted phenethylamide center, a carboxylic acid and the presence of a Ph ring were found to be assocd. with increased affinity at both CCK-A and CCK-B receptors.

Benigni R, Richard AM. **QSARS of mutagens and carcinogens: Two case studies illustrating problems in the construction of models for noncongeneric chemicals**. Mutat Res 1996;371(1-2):29-

46.
BIOSIS COPYRIGHT: BIOL ABS. There is a strong motivation to develop QSAR models for toxicity prediction for use in screening, for setting testing priorities, and for reducing reliance on animal testing. Decisions must be made daily by toxicologists in governments and industry to direct limited testing resources to the most urgent public health problems, and to direct the types of chemical synthesis and product development efforts undertaken. This need has motivated attempts to construct general QSAR models (e.g., for rodent carcinogenicity), not tailored to congeneric series of chemicals. These various attempts have provided interesting and important scientific evidence; however, they have also shared a limited overall performance. The goal of this paper is to illustrate, by two unrelated actual examples of QSARs for mutagens and carcinogens, some fundamental problems relative to the application of general QSAR approaches to noncongeneric chemicals. Both examples consider data sets that are noncongeneric in a chemical structure and mechanism of action sense: in the first case, a mean mutagenic potency defined as an average over multiple genetic toxicity endpoints, and, in the second case, the NTP two-sexes, two species rodent carcinogenicity bioassay results for 280 carcinogens and noncarcinogens. The problems encountered with the QSAR analyses of these two cases indicate that a successful approach to the problem of QSAR modeling of noncongeneric data will need to consider the multidimensional nature of the problem in both a chemical and a biological sense. Since different chemical classes represent largely independent action mechanisms, some means for extracting local QSARs for constituent classes will be necessary. Alternatively, a general QSAR derived for a noncongeneric data set will need to be scrutinized and decomposed along chemical class lines in order to establish boundaries for application and confidence levels for prediction.

Bradbury SP, Mekenyan OG, Ankley GT. **Quantitative structure-activity relationships for polychlorinated hydroxybiphenyl estrogen receptor binding affinity: an assessment of conformer flexibility**. Environ Toxicol Chem 1996;15(11):1945-54.

Chambers PL, Chambers CM, Kennedy AC. **Validation with in vitro test systems of the quantitative structure activity relationships (QSARA) used for the prediction of toxicology of new fire retardants**. Polym Degrad Stab 1996;54(2-3):323-8.
CBAC COPYRIGHT: CHEM ABS Conventional in vivo toxicol. studies were proscribed under the terms of this project. Consequently, toxicol. predictions were based on SARs and QSARs and selected in vitro biol. tests. The TOPKAt program was used to provide ests. of: rat acute oral toxicity (LD50), rat max. tolerated dose (MTD), mouse inhalational toxicity (24 h LC50), eye and skin irritation, mutagenesis, carcinogenesis, teratogenesis, aerobic biodegrdn., fish 96 h LC50, and Daphnia magna 48 h LC50. The toxicol. activity of all the chems. was also evaluated using Microtox, Mutatox. Some evidence of mutagenic and carcinogenic potential was detected. Good correlation was shown between the prediction of some of the exptl. parameters of toxicity. TOPKAT predicted bis(aminophenyl)methyl phosphine oxide to be very toxic. Expert judgement contradicted this finding. Exptl. it did not inhibit acetylcholinesterase which supported the hypothesis that the P=O moiety was hindered and hence inactive as an anticholinesterase.

Chan AW, Golec JM. **Prediction of relative potency of ketone protease inhibitors using molecular orbital theory**. Bioorg Med Chem 1996;4(10):1673-7.

CBAC COPYRIGHT: CHEM ABS MO calcns. were carried out on a series of model ketonic protease inhibitors. A comparison of the LUMO energy of the ketones in a variety of model heterocyclic ketone protease inhibitors shows a correlation with the electrophilicity of the carbonyl and the sigma1 exptl. data. It is also obsd. that the more neg. charge on the nitrogen atom in the heterocyclic ring the greater its potential as a hydrogen bond acceptor. The results of this study provide a simple means of predicting relative inhibitor potency and is therefore of use both to medicinal chemists designing protease inhibitors and in QSAR studies.

Chilmonczyk Z, Bogdal M, Mazgajska M, Cybulski J, Lewandowska U. **Structure-activity relationship in a series of new 1-(2-pyrimidinyl)piperazine derivatives with hypnotic activity**. Pol J Pharmacol 1996;48(4):431-40.
CBAC COPYRIGHT: CHEM ABS Prepn., biol. properties and QSAR of new derivs. of 1-[4-(2-pyrimidinyl)-1-piperazinyl]-1,3-butandione (11-13, and 15-18) and 3-[4-(2-pyrimidinyl)-1-piperazinyl]-3-oxopropanoate (20-22).

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

Domine D, Devillers J, Wienke D, Buydens L. **ART 2-A for optimal test series design in QSAR**. J Chem Inf Comput Sci 1997;37(1):10-7.
CBAC COPYRIGHT: CHEM ABS The family of adaptive resonance theory (ART)-based systems concerns distinct artificial neural networks for unsupervised and supervised clustering anal. Among them, the ART 2-A paradigm presents numerous strengths for data anal. After a rapid presentation of the ART 2-A theory and algorithmic information, the usefulness of this neural network for the selection of optimal test series is estd. The results are compared with those obtained from hierarchical cluster anal. and visual mapping methods. The advantages and drawbacks of each method are discussed. ART 2-A represents a new useful nonlinear statistical tool for QSAR and drug design.

Duffy JC, Dearden JC, Rostron C. **A QSAR study of anti-inflammatory N-arylanthranilic acids**. J Pharm Pharmacol 1996;48(9):883-6.
CBAC COPYRIGHT: CHEM ABS A detailed quant. structure-activity relationship (QSAR) anal. of a series of 112 anti-inflammatory N-arylanthranilic acids has been performed to det. which physicochem. properties of these compds. are responsible for.

Flocco MM, Carrell HL, Harvey RG, Zacharias DE, Glusker JP. **The structure of a coumarin derivative related to the carcinogen benz[a]anthracene**. Carcinogenesis 1996;17(10):2245-8.
The three-dimensional structure of 3-methyl-2H-anthra[1,2-b]pyran-2-one, an anticarcinogenic

coumarin related to the carcinogen benz[a]anthracene, has been determined by X-ray diffraction techniques. The molecule, apart from hydrogen atoms in the methyl group, is flat, the maximum deviation from its least squares best plane being 0.13 angstroms. The carbonyl C=O bond length is normal [1.206(1) angstroms] and the bonding throughout the molecule indicates localization of double bonds within the coumarin ring, but some delocalization of electrons in the other rings. Molecules pack in planes parallel to each other, the coumarin ring oxygen atom lying between two aromatic rings of other coumarin molecules. The bulky methyl groups are not involved in such stacking, while the carbonyl groups attract C-H groups in neighboring molecules by way of C-H...O interactions. These are the types of interactions that such coumarins could make if they bound to hydrophobic areas in biological macromolecules.

Franke R. **Chemometric methods in drug design: tale or tool?** Bioact Compd Des 1996;89-98.
CBAC COPYRIGHT: CHEM ABS A review with 8 refs. Classical QSAR methods still play an important role in drug design and will gain in importance with the advent of high-throughput screening systems. They can provide information about the mechanism of action provided that certain conditions are met. One condition is the correctness of parameters used. Examples for necessary corrections for computed log P values are presented. Another important issue are colinearities which can be avoided by series design techniques. QSARs have provided certain rules which can be very helpful in the development of drugs. A typical example is the bioisosteric replacement of substituents to improve pharmacokinetic properties. Very important but greatly neglected in QSAR work are activity-activity relationships. Several examples are presented including relationships between results from in vitro and in vivo tests, multivariate relationships from batteries of tests, and structure-selectivity relationships.

Gamberger D, Horvatic D, Sekusak S, Sabljic A. **Applications of experts' judgement to derive structure-biodegradation relationships**. Environ Sci Poll Res Int 1996;3(4):224-8.
BIOSIS COPYRIGHT: BIOL ABS. The experts' judgement data on microbial degradation were used to develop the first general QSAR biodegradability model (Boethling and Sabljic, 1989) which is composed of a set of structural descriptors and a set of quantitative rules. Its evaluation and validation with experimental biodegradation data clearly show that the developed model gives a realistic and reliable account of structure-biodegradability relationship for organic chemicals. The same set of experts judgement data was used to develop structure-biodegradation rule by the application of an inductive machine learning method. An improved structure-biodegradation rule was derived from a larger training set of 160 chemicals, i.e. the combined experts' judgement and evaluated experimental biodegradation data. This rule has good predictive ability and discloses logical dependencies between structural features that have a strong influence on biodegradation of organic chemicals. Thus, the understanding of biodegradation processes will benefit from developed rule.

Grigorov M, Weber J, Tronchet JM J, Jefford CW, Milhous WK, Maric D. **A QSAR study of the antimalarial activity of some synthetic 1,2,4-trioxanes**. J Chem Inf Comput Sci 1997;37(1):124-30.
CBAC COPYRIGHT: CHEM ABS The antimalarial activity of a series of synthetic 1,2,4-trioxanes is correlated with mol. structure by using a pharmacophore search method (CATALYST). The technique is shown to have predictive accuracy and confirms that docking between an active trioxane and the receptor, heme, is the crucial step for drug action.

Hannongbua S, Lawtrakul L, Sotriffer CA, Rode BM. **Comparative molecular field analysis of HIV-1 reverse transcriptase inhibitors in the class of 1[(2-hydroxyethoxy)-methyl]-6-(phenylthio) thymine**. Quant Struct Act Relat 1996;15(5):389-94.
CBAC COPYRIGHT: CHEM ABS A Comparative Mol. Field Anal. of 40 HEPT (1-[(2-Hydroxyethoxy)-methyl]-6-(phenylthio)thymine) analogs was performed. The 3D-QSAR study correlates steric and electrostatic fields of the compds. with HIV-1 RT inhibition, resulting in a model which has a high predictive ability. CoMFA results revealed that steric interaction explains a majority (70%) of the variance in the data. Using only steric interaction energy in the analyses provided a better predictive model.

Hu F, Wang S, Xiang J, Liang B, Sakai M, Muramatsu Y, Li Z. **A novel method for rejecting ill-conditioned parameters in description matrix of QSAR**. Hunan Daxue Xuebao 1996;23(3):59-64. (Chi)
CBAC COPYRIGHT: CHEM ABS In quant. structure-activity relationship (QSAR) studies, the correlation matrix of description parameters xi is ill-conditioned (pathol.) when these parameters are seriously correlated. An then the QSAR regression equation between activity y and variable xi obtained is unstable and unuseful. A novel method is developed for obtaining a stable QSAR equation. A proper crit. value alphaepsilon (0,1) is given. The parameters involved are found and some of them are rejected, whose correlation coeff. is larger than or equal to the crit. value .alpha.. By using the remaining parameters and stepwise regression, a stable and useful QSAR equation can be obtained. The presented novel method can widely be used in QSAR and mol. design.

Hyde RM, Hersey A. **Screening and rational drug design - competitive or complementary?** Bioact Compd Des 1996;37-45.
CBAC COPYRIGHT: CHEM ABS A review with 9 refs. In order to exploit the potential of an existing library of compds. for lead-hunting, the need for a robotic compd. handling system had been recognized. In setting up such a system, it was important to re-evaluate the implications for the so-called rational drug design techniques such as computational chem. and QSAR. It is proposed that the increased availability of automated procedures is not competitive with the rational approaches but accentuates their importance. New responses are required at all stages from the selection of compds. for screening through to the processes of selecting and optimizing robust leads.

Ivanciuc O. **Artificial neural networks applications. Part 2. Using theoretical descriptors of molecular structure in quantitative structure-activity relationships: analysis of the inhibition of dihydrofolate reductase**. Rev Roum Chim 1996;41(7-8):645-52.
CBAC COPYRIGHT: CHEM ABS Quant. structure-activity relationships (QSAR) were employed to correlate structure to activity mainly by the use of multiple linear regression (MLR). A general problem of MLR models, i.e., the lack of nonlinear mapping, is resolved by the use of a new approach in computational chem.: artificial neural networks (ANN). A comparison is made between the ability of MLR and ANN to predict the inhibitory potencies of substituted s-triazine derivs. on chicken liver dihydrofolate reductase; as chem. structure characteristics, 3 theor. descriptors were used. Comparing the statistics of the 2 models, ANN performs better than MLR, providing accurate predictions of the biol. activities of the s-triazine derivs.

Kamenska V, Ivanov T, Nedyalkova Z, Petkov O, Lutekov G, Taskov M, Nikolov G, Mekenyan O. **Computer design and syntheses of antiulcer compounds. 1st Communication. N-:3-[3-(1-piperidinomethyl)phenoxy]propyl:amines and benzamides**. Arzneimittelforschung 1996;46 (11):1090-5.
CBAC COPYRIGHT: CHEM ABS To develop new antiulcer agents, a QSAR study on in vitro (pA2) and in vivo histamine H2-receptor antagonistic activity of a series of N-:3-[3-(1-piperidinomethyl) phenoxy]propyl:amines was carried. PA2 increases with the decrease (increase) of electron donator (acceptor) properties of mols., particularly at the NH-reaction site. The finding is consistent with the assumption for an increase of histamine H2-receptor activity of the antagonists with their ability to form H-bonds with the receptor through NH groups. The correlations with hydrophobicity and related topol. indexes are consistent with the hypothesis that log P should indirectly reflect receptor interactions. In addn. a series of N-:3-[3-(1-piperidinomethyl)phenoxy]propyl:benzamides were synthesized. The theor. predicted in vitro activities of these compds. were in accordance with in vivo tests :percent of inhibition of gastric juice and acid output [mEq/H+/3 h]:.

Mabilia M, Fioravanzo E. **Non-peptide angiotensin II receptor antagonists: PCA & PLS analyses employing different types of 3D molecular descriptors**. Bioact Compd Des 1996;127-36.
CBAC COPYRIGHT: CHEM ABS Different three-dimensional (3D) mol. descriptors were generated for a set of angiotensin II receptor antagonists with the aim to: a. establish quant. structure-activity relationships (QSAR) by means of Principal Component Anal. (PCA) and Partial Least-Squares (PLS) methods, b. compare the modeling and predictive capabilities of the different types of descriptors considered, c. establish the effect of exptl. design techniques to select objects (mols.) and variables (descriptors), with respect to the predictive power of the QSAR models derived.

McKinney JD. **Reactivity parameters in structure-activity relationship-based risk assessment of chemicals**. Environ Health Perspect 1996;104(8):810-6.
New approaches to the risk assessment process are needed that might be more definitive and satisfying to the scientific community, interest groups, and the public at large. This commentary examines an alternative approach that is based on understanding the relationships of chemical structure and reactivity properties to the toxicokinetic behavior of chemicals in biological systems. This approach is based on the likelihood that there is a limited number of triggering (reactivity) mechanisms by which chemicals can express their toxicity at the molecular level. The fundamental importance of electrophilic character of chemicals as a determinant of their critical molecular reactivities and interactions with biological material in the expression of toxicity is supported. Such an approach also takes advantage of the maturing field of theoretical/computational chemistry in understanding important molecular recognition and reactivity processes (both qualitatively and quantitatively) for chemicals that can underlie their biological/toxicological activity. A process that permits assessment of reaction equivalents delivered to biological systems may hold promise for grouping chemicals by common triggering mechanisms with clearly delineated toxicological endpoints.

Moriguchi I, Hirano H, Hirono S. **Prediction of the rodent carcinogenicity of organic compounds from their chemical structures using the FALS method**. Environ Health Perspect 1996;104(Suppl 5):1051-8.
BIOSIS COPYRIGHT: BIOL ABS. Fuzzy adaptive least-squares (FALS), a pattern recognition method

recently developed in our laboratory for correlating structure with activity rating, was used to generate quantitative structure-activity relationship (QSAR) models on the carcinogenicity of organic compounds of several chemical classes. Using the predictive models obtained from the chemical class-based FALS QSAR approach, the rodent carcinogenicity or noncarcinogenicity of a group of organic chemicals currently being tested by the U.S. National Toxicology Program was estimated from their chemical structures.

Mracec M, Mracec M, Kurunczi L, Nusser T, Simon Z, Naray-Szabo G. **QSAR study with steric (MTD), electronic and hydrophobicity parameters on psychotomimetic phenylalkylamines**. Theochem 1996;367:139-49.
CBAC COPYRIGHT: CHEM ABS Multiple linear regression anal. has been used to identify the most important properties relevant to psychotomimetic activity displayed by 37 phenylalkylamines. Using the minimal topol. differences (MTD) parameter, lipophilicity (log P, calcd. by using pi Hansch substituent.

Munro IC, Ford RA, Kennepohl E, Sprenger JG. **Correlation of structural class with no-observed-effect levels: a proposal for establishing a threshold of concern**. Food Chem Toxicol 1996;34(9):829-67.
The relationship between chemical structure and toxicity was explored through the compilation of a large reference database consisting of over 600 chemical substances tested for a variety of endpoints resulting in over 2900 no-observed-effect levels (NOELs). Each substance in the database was classified into one of three structural classes using a decision tree approach. The resulting cumulative distributions of NOELs for each of the structural classes differed significantly from one another, supporting the contention that chemical structure defines.

Muresan S, Dragos D, Bologa C, Ciubotariu D. **Contributions to quantitative structure-activity relationship (QSAR) studies. The minimal steric difference (MTD and MSD) methods and application**. Stud Univ Babes Bolyai Chem 1994;39(1-2):63-9.
CBAC COPYRIGHT: CHEM ABS Original Timisoara methods for QSAR studies: MTD (Minimal Topol. Difference) and MSD (Minimal Steric Difference) are detailed and updated. The multiconformational MTD method is applied on a set of 25 acetic esters vs. the acetylcholinesterase hydrolysis rates, and results are discussed.

Nguyen-Cong V, Van Dang G, Rode BM. **Using multivariate adaptive regression splines to QSAR studies of dihydroartemisinin derivatives**. Eur J Med Chem 1996;31(10):797-803.

Okey RW, Stensel HD. **A QSAR-based biodegradability model: A QSBR**. Water Res 1996;30(9): 2206-14.
BIOSIS COPYRIGHT: BIOL ABS. A microbial biodegradability predictive model has been developed using groups and molecular indices as molecular descriptors. The model contains 12 variables. The model was calibrated and later validated with a data set developed from biodegradation studies of acclimated activated sludge metabolism. It also successfully predicted the biodegradation rate of several other substances with one exception within the limits established by a study of a second.

Poroikov VV, Filimonov DA, Stepanchikova AV, Budunova AP, Shilova EV, Rudnitskikh AV, Selezneva TM, Goncharenko LV. **[Optimization of synthesis and pharmacological study of substances based on computer prediction of their biological activity spectrum]**. Khim Farm Zh 1996;30(9):20-3. (Rus)

Puri RD, Mirgal SV, Ramaa CS, Kulkarni VM. **Chromatographically derived hydrophobicity parameters in QSAR analysis of diarylsulfone analogs**. Indian J Chem 1996;35b(12):1271-4.
CBAC COPYRIGHT: CHEM ABS 4-Amino-4'-substituted diphenylsulfones were synthesized and tested for antileprotic activity. The values of the parameters Rm and log K were estd. using reverse phase TLC (RPTLC) and reverse phase HPLC (RPHPLC), resp., and correlated individually with calcd. pi and mol. connectivity (chi) values in a typical Hansch anal. Results indicated that log K is a better descriptor of lipophilicity than Rm in the present homologous series of compds. The mol. connectivity index appeared to be a poor descriptor of the lipophilic characteristic of these compds. Quant. relationships between the various physicochem. parameters and biol. activity were analyzed using linear regression anal. It was found that the electronic effect of a substituent is more contributive for inhibitory potency than lipophilicity.

Sakamoto Y, Aoki T, Ohshima S. **Relationship between the 13C-NMR chemical shift and the carcinogenic activity of benz[a]anthracenes**. Chem Pharm Bull (Tokyo) 1996;44(2):424-8.
A thorough NMR spectroscopic investigation was made on 7,12-dimethylbenz[a]anthracene (1) and the 1H- and 13C-NMR spectra were completely assigned. We compared the 13C-NMR chemical shifts (delta) of three benz[a]anthracenes, including compound 1, whose carcinogenicities vary largely, and investigated the relationship of the delta and carcinogenicity. It was found that the delta values at the C1, C2, and C6 positions differed significantly between the carcinogenic and noncarcinogenic compounds.

So S, Karplus M. **Genetic neural networks for quantitative structure-activity relationships: improvements and application of benzodiazepine affinity for benzodiazepine/GABAA receptors**. J Med Chem 1996;39(26):5246-56.
CBAC COPYRIGHT: CHEM ABS A novel tool, called a genetic neural network (GNN), has been developed for obtaining quant. structure-activity relationships (QSAR) for high-dimensional data sets. The GNN method uses a neural network to correlate activity with descriptors that are preselected by a genetic algorithm. To provide an extended test of the GNN method, the data on 57 benzodiazepines given by D. J. Maddalena and G. A. R. Johnston (1995) have been examd. with an enhanced version of GNN, and the results are compared with the excellent QSAR of these authors. The problematic steepest descent training has been replaced by the scaled conjugate gradient algorithm. This leads to a substantial gain in performance in both robustness of prediction and speed of computation. The cross-validation GNN simulation and the subsequent run based on an unbiased and more efficient protocol led to the discovery of other 10-descriptor QSARs that are superior to the best model of the previous authors based on backward elimination selection and neural network training.

Tanaka A, Fujiwara H. **Quantitative structure-activity relationship study of fibrinogen inhibitors, [[4-(4-amidinophenoxy)butanoyl]aspartyl]valine (FK633) derivatives, using a novel hydrophobic descriptor**. J Med Chem 1996;39(25):5017-20.
CBAC COPYRIGHT: CHEM ABS The authors recently reported a novel hydrophobic descriptor for

quant. structure-activity relationship (QSAR) studies, the logarithm of the partition coeff. micelle/water (log Pmw), which is easily detd. by a HPLC system and is thought to be a descriptor for a compd.'s affinity to a biomembrane. QSAR studies were carried out using log Pmw on the antiplatelet activities of novel fibrinogen inhibitors, [[4-(4-amidinophenoxy)butanoyl]aspartyl]valine (FK633) derivs., which resulted in a quadratic curve with a good correlation coeff. (n = 12, s = 0.368, F = 14.1**, r = 0.871), indicating that a suitable membrane affinity of the fibrinogen inhibitors is vital for their inhibitory activities. QSAR studies using STERIMOL parameters and/or CLOGP values were unsuccessful.

Ter Haar E, Rosenkranz HS, Hamel E, Day BW. **Computational and molecular modeling evaluation of the structural basis for tubulin polymerization inhibition by colchicine site agents**. Bioorg Med Chem 1996;4(10):1659-71.
CBAC COPYRIGHT: CHEM ABS The computer-automated structure evaluation programs MultiCASE and CASE were used to perform a quant. structure-activity relationship study on tubulin polymn. inhibitors. A learning set of 536 chems. (202 active, 27 marginal, and 307 inactive), built using IC50 values for inhibition of tubulin polymn. or mitosis from this and previous studies, was used for artificial intelligence self-teaching. The algorithms successfully predicted the activity of agents in the learning set with >90% accuracy. Seventeen MultiCASE and 12 CASE (mostly included in the MultiCASE set) biophores (substructures significantly correlated with activity) were identified with a probability >0.95. Here the authors present the biophores of podophyllotoxins, colchicinoids, and certain combretastatins, each examd. for structure-activity relationships. For the podophyllotoxins and colchicinoids in the learning set, the correlations between obsd. and predicted potencies were >0.85. The algorithms recognized the importance of several known site, electronic, and steric effects in the 2 classes. A predictive QSAR (R2 = 0.98) was developed for combretastatin A-2 and dihydrocombretastatin analogs. The MultiCASE/CASE analyses were used in combination with mol. models to study relative orientations of colchicine, podophyllotoxin, combretastatin A-4, and steganacin at the colchicine site. This resulted in a new hypothesis, consistent with extensive published exptl. data, in which the C-ring and part of the B-ring of colchicine overlap with the A- and B-rings of podophyllotoxin. Consequently, the trimethoxyphenyl rings of colchicine and podophyllotoxin occupied different regions of space, each pointing out from a hydrophobic core occupied by the overlapping biophores. The mol. model of the highly potent combretastatin A-4 could fit into the model binding site in .gtoreq.3 different ways. The developed QSARs were used to identify the potent microtubule stabilizer discodermolide. Its identification, in concert with recently reported findings, suggest potential overlap in the colchicine and paclitaxel binding sites on tubulin.

Waller CL, Oprea TI, Chase K, Park HK, Korach KS, Laws SC, Wiese TE, Kelce WR, Gray LJ. **Ligand-based identification of environmental estrogens**. Chem Res Toxicol 1996;9(8):1240-8.
BIOSIS COPYRIGHT: BIOL ABS. Comparative molecular field analysis (CoMFA), a three-dimensional quantitative structure-activity relationship (3D-QSAR) paradigm, was used to examine the estrogen receptor (ER) binding affinities of a series of structurally diverse natural, synthetic, and environmental chemicals of interest. The CoMFA/3D-QSAR model is statistically robust and internally consistent, and successfully illustrates that the overall steric and electrostatic properties of structurally diverse ligands for the estrogen receptor are both necessary and sufficient to describe the binding affinity. The ability of the model to accurately predict the ER binding affinity of an external test set of

molecules suggests that structure-based 3D-QSAR models may be used to supplement the process of endocrine disruptor identification through prioritization of novel compounds for bioassay. The general application of this 3D-QSAR model within a toxicological framework is, at present, limited only by the quantity and quality of biological data for relevant biomarkers of toxicity and hormonal responsiveness.

Wieland T. **The use of structure generators in predictive pharmacology and toxicology**. Arzneimittelforschung 1996;46(2):223-7.
The analysis of pharmacological effects of chemical compounds often requires expensive of database searches. But in many cases, some of the most useful (or toxic) substances are overlooked because they are missing in the experiment schedule or in the database etc. In the recent years quantitative structure-activity relationship (QSAR) studies have shown several correlations of physiochemical properties and topological information indices. In the present paper, the use of an automatic structure generator is studied for detecting compounds with maximal activity by (carefully) extrapolating these correlations. So assumptions of optimal activity can be easier evidenced or discarded. The ideas are outlined on barbiturates and monoketones.

Xie G, Li B, Zhou J. **QSAR study on larvicidal 1-(substituted benzoyl)-2-benzoyl-1-tert-butylhydrazines**. Jisuanji Yu Yingyong Huaxue 1996;13(3):188-92.

Yan Z, Huang W, Peng S, Hua W, Ji N. **[Studies on the bioactivity and QSAR of some 4-acyloxybenzopyrans]**. Zhongguo Yaoke Daxue Xuebao 1996; 27(7):385-94. (Chi)
CBAC COPYRIGHT: CHEM ABS Based on the structure-activity relationships of some benzopyran potassium channel openers, 19 4-acyloxybenzopyrans were designed, synthesized and evaluated for their bioactivity in vivo and in vitro. QSAR was studied by stepwise discriminant anal. on the basis of calcd. structural parameters and bioactivities (vasodilation in vitro and hypotension in vivo).

Zhang D. **[QSAR studies of mono-substituted benzene derivatives - application of molecular surface area and the surface area of active groups]**. Huanjing Huaxue 1996;15(6):536-40. (Chi)
CBAC COPYRIGHT: CHEM ABS Structure-toxicity relationship of 30 mono-substituted benzene derivs. were studied. Because variety of substituents and defference of their properties, when electronic property obtained by quantum chem. computations, topol. indexes and some empirical parameters were used as descriptors, the good correlation could not be attained. Then, a surface area of the active group was introduced, together with a mol. surface area and a third-order mol. shape index, which reveal a total mol. property and shape, the regression equation obtained reflects better the relationship between structers and toxicities. It is also showed that substituents could play an important role in the toxic effect of mono-substituted benzene derivs.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Allende ML, Amsterdam A, Becker T, Kawakami K, Gaiano N, Hopkins N. **Insertional mutagenesis in zebrafish identifies two novel genes, pescadillo and dead eye, essential for embryonic development** . Genes Dev 1996;10(24):3141-55.

Recently our laboratory described an efficient method for generating retroviral provirus insertions in the zebrafish germ line, and we showed that provirus insertions induce embryonic mutations at a frequency of roughly one mutant per 70 insertions. To date we have isolated four insertional mutants and, using the proviruses as a molecular tag, have cloned the genes disrupted in three of them. The proviruses in all three mutants lie within or just 5' of the first coding exon, point in the opposite transcriptional orientation from the gene, and disrupt transcription. Here we present a molecular characterization of two genes identified by this method and describe the associated mutant phenotypes. The pescadillo (pes) gene is predicted to encode a protein of 582 amino acids with no recognizable functional motifs, which is highly conserved from yeast to humans. pes mRNA is expressed widely and dynamically during the first 3 days of embryogenesis. Prominent sites of expression are the eyes and optic tectum on day 1, the fin buds, liver primordium, and gut on day 2, and the branchial arches on day 3. Beginning at day 3 of embryogenesis, pes mutant embryos exhibit small eyes, a reduced brain and visceral skeleton, shortened fins, and a lack of expansion of the liver and gut, and then die on the sixth day of development. The dead eye (dye) gene encodes a protein of 820 amino acids that is homologous to genes of unknown function in human, mouse, and Xenopus, and that has weak homology with the yeast NIC96 (nucleoporin-interacting component) gene. dye mutants can be recognized on day 2 of embryogenesis by the presence of necrotic cells in the tectum and eyes. dye mutants die on day 5 of development. These results demonstrate the power of insertional mutagenesis in zebrafish for rapidly finding and characterizing novel genes essential for embryonic development. Using our current methodology, we estimate that our laboratory could screen approximately 25,000 insertions in 2-3 years, identifying perhaps 250-350 embryonic lethal genes. Assuming that all genes are accessible to proviral insertion, the wider application of this approach could lead to the rapid identification of the majority of genes that are required for embryonic development of this vertebrate.

Bantle JA, Finch RA, Burton DT, Fort DJ, Dawson DA, Linder G, Rayburn JR, Hull M, Kumsher-King M, Gaudet-Hull AM, et al. **FETAX interlaboratory validation study: Phase III-Part 1 testing**. J Appl Toxicol 1996;16(6):517-28.
BIOSIS COPYRIGHT: BIOL ABS. The Frog Embryo Teratogenesis Assay-Xenopus (FETAX) is a 96-h whole embryo developmental toxicity screening assay that can be used in ecotoxicology and in detecting mammalian developmental toxicants when an in vitro metabolic activation system is employed. A standardized American Society for Testing and Materials (ASTM) guide for the conduct of FETAX has been published, along with a companion atlas that aids in embryo staging and identifying malformations. As part of the ASTM process, a three-phase interlaboratory validation study was undertaken to evaluate the repeatability and reliability of FETAX. Seven different participants collaborated in the study. In Phase I, FETAX proved to be more repeatable and reliable than many bioassays. However, some excessive variation was observed in a few laboratories. An initial lack of assay experience by some technicians caused variation. Phase II showed far less intra- and interlaboratory variability than Phase I. Non-teratogens showed the most consistent results, while more variability was observed for the two teratogens tested. Interlaboratory coefficient of variation values for all endpoints ranged from 7.3 to 54.7. Phase III-Part 1, using coded samples and test concentration ranges selected by each laboratory, showed results similar to Phase I. Analysis of the causes of variation suggested that some technicians judged some embryos to be malformed while others consistently judged similar embryos as normal. Concentration ranges tested by some of the laboratories varied greatly and a

new protocol for selecting concentrations for initial testing was written to reduce variation from this source. Testing to date suggests that FETAX is as repeatable and reliable as other standard bioassays.

Beckers J, Gerard M, Duboule D. **Transgenic analysis of a potential Hoxd-11 limb regulatory element present in tetrapods and fish**. Dev Biol 1996;180(2):543-53.
CBAC COPYRIGHT: CHEM ABS Genes of the HoxD complex related to the Drosophila Abd-B gene are involved in the morphogenesis of vertebrate paired appendages. Hoxd-11, for instance, is necessary in combination with other Hox genes for the proper development of different parts of the tetrapod limbs. Sequence comparisons between the mouse, chicken, and zebrafish Hoxd-11 loci have revealed the conservation of several blocks of DNA sequence which may be of importance for the regulation of Hoxd-11 expression. We have used transgenic mice to show that one of these conserved elements specifically drives expression in a proximal-posterior part of developing forelimbs. Prodn. of mice transgenic for a full fish Hoxd-11 construct as well as for mouse-fish Hoxd-11 chimeric constructs shows that the fish counterpart of this sequence is able to elicit expression in mouse forelimbs as well, though in a slightly different domain. However, this fish element requires the presence of the mouse promoter and does not work in its own context. These results are discussed in light of both the control of Hoxd gene expression during limb development and the use of a comparative interspecies approach to understand the regulation of genes involved in vertebrate development.

Christian MS. **Review of reproductive and developmental toxicity of 1,3-butadiene**. Toxicology 1996;113(1-3):137-43.
BIOSIS COPYRIGHT: BIOL ABS. Toxic doses of 1,3-butadiene (BD) have been reported to cause reproductive and/or developmental toxicity. Regardless of the strain used, mice were always affected by BD at lower doses than rats, an expected observation, based on well recognized differences in pharmacokinetic (PK) parameters in these two species. Because the mouse is particularly sensitive to BD in comparison with other laboratory species, and there are important functional and anatomical differences between humans and mice, the NOELs and LOELs identified for BD for various reproductive endpoints in mice may not be relevant to human reproductive risk. In mice, the LOELs for reproductive endpoints include developmental toxicity at 200 ppm, genotoxic effects at 500 ppm (mouse spot test), ovarian atrophy in females at 6.25 ppm (carcinogenicity study), reduced testicular weights at 200 ppm and testicular atrophy at 625 ppm BD in males (carcinogenicity studies), low incidences of abnormal sperm heads at 1000 and 5000 ppm BD (sperm head morphology study), small reversible increases in resorption at 1250/1300 ppm or 5000 ppm (dominant lethal studies), and other possible sequelae of genotoxicity resulting from exposure of male mice at 12.5 ppm BD and higher (dominant lethal study). When available, the much higher NOELs and LOELs of other species tested for the same endpoints should be considered. For example, maternal and developmental NOELs for BD in the rat were 200 and 1000 ppm, respectively, and 40 ppm in the mouse. Likewise, exposure of cohabited pairs of rats, guinea pigs and rabbits or of female dogs to BD concentrations as high as 6700 ppm for 8 months did not impair fertility or cause testicular or ovarian atrophy in these species. Thus, consideration of these remarkable species-dependent differences in toxicity is necessary. In addition, there are alternative scientific interpretations for some of the mouse studies and this review attempts to address these areas. For example, it may be incorrect to categorize results indicating weak in vivo genotoxic effects in male mice (sperm head morphology and dominant lethal studies) at 12.5 ppm BD

and higher as reproductive effects because concentrations of BD as high as 5000 ppm did not affect mating, fertility or live litter sizes, even in this sensitive species. Similarly, it may be inappropriate to identify the ovary as a target organ for reproductive risk since the ovarian atrophy in mice was identified after completion of the normal reproductive life and after more. than 15 months of exposure. Neither ovarian nor testicular atrophy occurred in Sprague-Dawley rats after exposure to BD concentrations as high as 8000 ppm for 105 (females) or 111 (males) weeks.

Driever W, Solnica-Krezel L, Schier AF, Neuhauss SC, Malicki J, Stemple DL, Stainier DY, Zwartkruis F, Abdelilah S, Rangini Z, et al. **A genetic screen for mutations affecting embryogenesis in zebrafish**. Development 1996;123:37-46.
Systematic genome-wide mutagenesis screens for embryonic phenotypes have been instrumental in the understanding of invertebrate and plant development. Here, we report the results from the first application of such a large-scale genetic screening to vertebrate development. Male zebrafish were mutagenized with N-ethyl N-nitrosourea to induce mutations in spermatogonial cells at an average specific locus rate of one in 651 mutagenized genomes. Mutations were transmitted to the F1 generation, and 2205 F2 families were raised. F3 embryos from sibling crosses within the F2 families were screened for developmental abnormalities. A total of 2337 mutagenized genomes were analyzed, and 2383 mutations resulting in abnormal embryonic and early larval phenotypes were identified. The phenotypes of 695 mutants indicated involvement of the identified loci in specific aspects of embryogenesis. These mutations were maintained for further characterization and were classified into categories according to their phenotypes. The analyses and genetic complementation of mutations from several categories are reported in separate manuscripts. Mutations affecting pigmentation, motility, muscle and body shape have not been extensively analyzed and are listed here. A total of 331 mutations were tested for allelism within their respective categories. This defined 220 genetic loci with on average 1.5 alleles per locus. For about two-thirds of all loci only one allele was isolated. Therefore it is not possible to give a reliable estimate on the degree of saturation reached in our screen; however, the number of genes that can mutate to visible embryonic and early larval phenotypes in zebrafish is expected to be several-fold larger than the one for which we have observed mutant alleles during the screen. This screen demonstrates that mutations affecting a variety of developmental processes can be efficiently recovered from zebrafish.

Fort DJ, Stover EL, Propst T, Group TS, Hull MA, Bantle JA. **Evaluation of the developmental toxicity of theophylline, dimethyluric acid, and methylxanthine metabolites using Xenopus**. Drug Chem Toxicol 1996;19(4):267-78.
BIOSIS COPYRIGHT: BIOL ABS. The developmental toxicities of theophylline and theophylline metabolites were evaluated using FETAX (Frog Embryo Teratogenesis Assay - Xenopus). Young X. laevis embryos were exposed to theophylline, 1-methylxanthine, 3-methylxanthine, or 1,3-dimethyluric acid in each of two separate concentration-response experiments with and without an exogenous metabolic activation system (MAS) and/or inhibited MAS. The MAS was treated with carbon monoxide (CO), cimetidine (CIM), or ellipticine (ELL) to selectively modulate cytochrome P-450 activity. Addition of the MAS and CIM-MAS reduced the developmental toxicity of theophylline. Addition of the ELL- or CO-inhibited MAS did not reduce the developmental toxicity of theophylline. Addition of the intact MAS did not alter the developmental toxicity of 1-methyl- or 3-methylxanthine which were slightly more developmentally toxic on an equimolar basis than theophylline itself. 1,3-dimethyluric

acid was not developmentally toxic at maximum soluble concentrations in 1% (v/v) DMSO. Results from these studies suggested that P-450, specifically ELL-inhibited P-450 (aryl hydrocarbon hydroxylase) may have been responsible for detoxification of theophylline and that 1,3 dimethyluric acid represented the primary detoxification metabolite of theophylline.

Gao D, Li Z, Murphy T, Sauerbier W. **Structure and transcription of the gene for translation elongation factor 1 subunit alpha of zebrafish (Danio rerio)**. Biochim Biophys Acta 1997;1350(1):1-5.
CBAC COPYRIGHT: CHEM ABS The zebrafish gene for translation elongation factor 1alpha (EF1alpha) was isolated from a phage Lambda genomic library, and its sequence and structure were detd. The authors found one gene copy of EF1alpha per haploid set of chromosomes and no processed pseudogenes. A highly active promoter region was localized to a 277 bp PstI/PvuII fragment beginning 240 bp upstream from the tsp, but no transcription enhancing, or silencing activity was obsd. within 1 kbp upstream, or downstream from the promoter. Expression of EF1alpha appears to be developmentally regulated

Haffter P, Granato M, Brand M, Mullins MC, Hammerschmidt M, Kane DA, Odenthal J, Van Eeden FJ, Jiang YJ, Heisenberg CP, et al. **The identification of genes with unique and essential functions in the development of the zebrafish, Danio rerio**. Development 1996;123:1-36.
In a large-scale screen, we isolated mutants displaying a specific visible phenotype in embryos or early larvae of the zebrafish, Danio rerio. Males were mutagenized with ethylnitrosourea (ENU) and F2 families of single pair matings between sibling F1 fish, heterozygous for a mutagenized genome, were raised. Egg lays were obtained from several crosses between F2 siblings, resulting in scoring of 3857 mutagenized genomes. F3 progeny were scored at the second, third and sixth day of development, using a stereomicroscope. In a subsequent screen, fixed embryos were analyzed for correct retinotectal projection. A total of 4264 mutants were identified. Two thirds of the mutants displaying rather general abnormalities were eventually discarded. We kept and characterized 1163 mutants. In complementation crosses performed between mutants with similar phenotypes, 894 mutants have been assigned to 372 genes. The average allele frequency is 2.4. We identified genes involved in early development, notochord, brain, spinal cord, somites, muscles, heart, circulation, blood, skin, fin, eye, otic vesicle, jaw and branchial arches, pigment pattern, pigment formation, gut, liver, motility and touch response. Our collection contains alleles of almost all previously described zebrafish mutants. From the allele frequencies and other considerations we estimate that the 372 genes defined by the mutants probably represent more than half of all genes that could have been discovered using the criteria of our screen. Here we give an overview of the spectrum of mutant phenotypes.

Haffter P, Nusslein-Volhard C. **Large scale genetics in a small vertebrate, the zebrafish**. Int J Dev Biol 1996;40(1):221-7.
The systematic isolation and characterization of mutants in Drosophila has enormously facilitated the analysis of molecular mechanisms underlying developmental pathways in the embryo. A similar approach is presently being used to study embryonic development of the zebrafish, which is becoming a mainstream model organism for vertebrate development. With its genetic versatility and sophisticated embryology, zebrafish offers the possibility to rapidly increase our knowledge of vertebrate development and add to what we have learned from other vertebrate model organisms.

Hammerschmidt M, Pelegri F, Mullins MC, Kane DA, Van Eeden FJ, Granato M, Brand M, Furutani-Seiki M, Haffter P, Heisenberg CP, et al. **Dino and mercedes, two genes regulating dorsal development in the zebrafish embryo**. Development 1996;123:95-102.
We describe two genes, dino and mercedes, which are required for the organization of the zebrafish body plan. In dino mutant embryos, the tail is enlarged at the expense of the head and the anterior region of the trunk. The altered expression patterns of various marker genes reveal that, with the exception of the dorsal most marginal zone, all regions of the early dino mutant embryo acquire more ventral fates. These alterations are already apparent before the onset of gastrulation. mercedes mutant embryos show a similar but weaker phenotype, suggesting a role in the same patterning processes. The phenotypes suggests that dino and mercedes are required for the establishment of dorsal fates in both the marginal and the animal zone of the early gastrula embryo. Their function in the patterning of the ventrolateral mesoderm and the induction of the neuroectoderm is similar to the function of the Spemann organizer in the amphibian embryo.

Heisenberg CP, Brand M, Jiang YJ, Warga RM, Beuchle D, Van Eeden FJ, Furutani-Seiki M, Granato M, Haffter P, Hammerschmidt M, et al. **Genes involved in forebrain development in the zebrafish, Danio rerio**. Development 1996;123:191-203.
We identified four zebrafish mutants with defects in forebrain induction and patterning during embryogenesis. The four mutants define three genes: masterblind (mbl), silberblick (slb), and knollnase (kas). In mbl embryos, the anterior forebrain acquires posterior forebrain characteristics: anterior structures such as the eyes, olfactory placodes and the telencephalon are missing, whereas the epiphysis located in the posterior forebrain is expanded. In slb embryos, the extension of the embryonic axis is initially delayed and eventually followed by a partial fusion of the eyes. Finally, in kas embryos, separation of the telencephalic primordia is incomplete and dorsal midline cells fail to form a differentiated roof plate. Analysis of the mutant phenotypes indicates that we have identified genes essential for the specification of the anterior forebrain (mbl), positioning of the eyes (slb) and differentiation of the roof plate (kas). In an appendix to this study we list mutants showing alterations in the size of the eyes and abnormal differentiation of the lenses.

Henry TR, Spitsbergen JM, Hornung MW, Abnet CC, Peterson RE. **Early life stage toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in zebrafish (Danio rerio)**. Toxicol Appl Pharmacol 1997; 142(1):56-68.
CBAC COPYRIGHT: CHEM ABS Toxicity and histopathol. of 2,3,7,8-tetrachlorodibenzo--dioxin (TCDD) in zebrafish (Danio rerio) early life stages was characterized from 12 to 240 h postfertilization (hpf) following waterborne exposure of newly fertilized eggs. TCDD did not increase egg mortality (0-48 hpf), nor did it affect time to hatching (48-96 hpf). Egg doses of 1.5 ng [3H]TCDD/g or greater elicited toxic responses in zebrafish larvae. Pericardial edema and craniofacial malformations were first obsd. at 72 hpf, followed by the onset of yolk sac edema (96 hpf) and mortality (132 hpf). At 240 hpf the ED50s for pericardial edema, yolk sac edema, and craniofacial malformations were 2.2, 2.1, and 1.9 ng [3H]TCDD/g egg, resp. The LD50, detd. at 240 hpf, was 2.5 ng [3H]TCDD/g egg. Severe hemodynamic changes, obsd. as slowed blood flow in vascular beds of the trunk, head, and gills and slowed heart rate, occurred in TCDD-treated zebrafish prior to or coincident with the onset of gross signs of toxicity. Histol. examn. of TCDD-treated zebrafish revealed a variety of epithelial tissue lesions including arrested gill development and ballooning degeneration and/or necrosis of the renal tubules, hepatocytes,

pancreas, and all major brain regions. Mesenchymal tissue lesions included s.c. edema in the head, trunk and yolk sac, edema of the pericardium and skeletal muscle, and underdevelopment of the swim bladder. This demonstration of zebrafish responsiveness to TCDD early life stage toxicity coupled with the considerable information on developmental biol. and genetics of zebrafish provides a foundation for future investigations into the mechanism of TCDD developmental toxicity.

Kane DA, Maischein HM, Brand M, Van Eeden FJ, Furutani-Seiki M, Granato M, Haffter P, Hammerschmidt M, Heisenberg CP, Jiang YJ, et al. **The zebrafish early arrest mutants**. Development 1996;123:57-66.
This report describes mutants of the zebrafish having phenotypes causing a general arrest in early morphogenesis. These mutants identify a group of loci making up about 20% of the loci identified by mutants with visible morphological phenotypes within the first day of development. There are 12 Class I mutants, which fall into 5 complementation groups and have cells that lyse before morphological defects are observed. Mutants at three loci, speed bump, ogre and zombie, display abnormal nuclei. The 8 Class II mutants, which fall into 6 complementation groups, arrest development before cell lysis is observed. These mutants seemingly stop development in the late segmentation stages, and maintain a body shape similar to a 20 hour embryo. Mutations in speed bump, ogre, zombie, specter, poltergeist and troll were tested for cell lethality by transplanting mutant cells into wild-type hosts. With poltergeist, transplanted mutant cells all survive. The remainder of the mutants tested were autonomously but conditionally lethal: mutant cells, most of which lyse, sometimes survive to become notochord, muscles, or, in rare cases, large neurons, all cell types which become postmitotic in the gastrula. Some of the genes of the early arrest group may be necessary for progression though the cell cycle; if so, the survival of early differentiating cells may be based on having their terminal mitosis before the zygotic requirement for these genes.

Kelsh RN, Brand M, Jiang YJ, Heisenberg CP, Lin S, Haffter P, Odenthal J, Mullins MC, Van Eeden FJ, Furutani-Seiki M, et al. **Zebrafish pigmentation mutations and the processes of neural crest development**. Development 1996;123:369-89.
Neural crest development involves cell-fate specification, proliferation, patterned cell migration, survival and differentiation. Zebrafish neural crest derivatives include three distinct chromatophores, which are well-suited to genetic analysis of their development. As part of a large-scale mutagenesis screen for embryonic/early larval mutations, we have isolated 285 mutations affecting all aspects of zebrafish larval pigmentation. By complementation analysis, we define 94 genes. We show here that comparison of their phenotypes permits classification of these mutations according to the types of defects they cause, and these suggest which process of neural crest development is probably affected. Mutations in eight genes affect the number of chromatophores: these include strong candidates for genes necessary for the processes of pigment cell specification and proliferation. Mutations in five genes remove part of the wild-type pigment pattern, and suggest a role in larval pigment pattern formation. Mutations in five genes show ectopic chromatophores in distinct sites, and may have implications for chromatophore patterning and proliferation. 76 genes affect pigment or morphology of one or more chromatophore types: these mutations include strong candidates for genes important in various aspects of chromatophore differentiation and survival. In combination with the embryological advantages of zebrafish, these mutations should permit cellular and molecular dissection of many aspects of neural

crest development.

Levy R, Eustache F, Pilikian S, Clavel C, Cordonier H, Benchaib M, Lornage J, Pinatel M, Guerin JF. **Effect of gastrin-releasing peptide on sperm functions**. Mol Hum Reprod 1996;2(11):867-72.
CBAC COPYRIGHT: CHEM ABS Male infertility can be related to defects in motility, capacitation, acrosome reaction, binding and penetration of the zona pellucida. While different in-vitro techniques (such as micromanipulation which is complicated and expensive) are available for the treatment of male infertility, several pharmacol. agents have been shown to increase fertilizing capacity under accurate exptl. conditions. Gastrin-releasing peptide (GRP, the mammalian homolog of the amphibian skin peptide bombesin) is present in the reproductive tract and expressed by the pregnant ovine endometrium prior to attachment and throughout the pregnancy. A bombesin-like peptide resulting from alternate splicing of the GRP gene in testis has been detected in primates. In this study, we have tested the ability of GRP to enhance human sperm functions such as motility, capacitation, zona binding and acrosome reaction. Anal. of sperm motility was performed with the ATS 20 computer-assisted semen anal. (CASA) system. Zona binding was analyzed using intact human unfertilized oocytes and selective labeling of spermatozoa with two fluorochromes. Our results did not show any pos. effect of GRP on these parameters under our exptl. conditions. However, when GRP at the concn. of 100 nM was added after ionophore treatment, the percentage of reacted cells increased significantly compared with situations where each agent was used alone. This led us to suppose that the role of bombesin in the different stages of fertilization might not exclude other unknown factors.

Lorenz R, Brueggemann R, Steinberg C Ew, Spieser OH. **Humic material changes effects of terbutylazine on behavior of zebrafish (Brachydanio rerio)**. Chemosphere 1996;33(11):2145-58.
BIOSIS COPYRIGHT: BIOL ABS. The long-term sublethal toxicity of the triazine herbicide terbutylazine (TBA; 1, 5, 50, 200 mug/l) alone and in presence of dissolved humic material (DHM; 2 mg TOC/l) on zebrafish (Brachydanio rerio) has been studied. The effect of the tested substances on behavior were investigated by recording and quantifying their spontaneous locomotor activity using the BehavioQuant system. Data were analysed by the Hasse diagram technique. For the parameter light-dark-preference, but not for the parameter inconstancy of motility, a clear concentration-effect-relationship for the groups under TBA as well as for the groups under TBA + DHM exposure could be found. Comparing TBA and TBA + DHM treated groups for both test parameters the presence of DHM increased the toxic effect of TBA on zebrafish for the preference and decreased for the motility. The main result is that the preference follows an additive principle, but not the motility. Hints for the influence of at least two different mechanisms controlling these behavioral parameters are discussed.

Malicki J, Schier AF, Solnica-Krezel L, Stemple DL, Neuhauss SC, Stainier DY, Abdelilah S, Rangini Z, Zwartkruis F, Driever W. **Mutations affecting development of the zebrafish ear**. Development 1996;123:275-83.
In a large scale screen for genetic defects in zebrafish embryogenesis we identified mutations affecting several aspects of ear development, including: specification of the otic placode, growth of the otic vesicle (otocyst), otolith formation, morphogenesis of the semicircular canals and differentiation of the otic capsule. Here we report initial phenotypic and genetic characterization of 20 of these mutations defining 13 independent loci. Embryos mutant at the quadro locus display abnormal specification of the otic placode. As revealed by dlx-3 expression, the otic field in the mutant embryos is smaller or split into

two fields. At later stages of development the ear of quadro mutants is frequently divided into two smaller, incomplete units. Four loci affect ear shape shortly after formation of the otic vesicle. All of them also display abnormal brain morphology. Mutations in five loci result in the absence of otolith formation; two of these also produce changes of ear morphology. Two loci, little richard and golas, affect morphology of the otic vesicle shortly before formation of the semicircular canals. In both cases the morphogenesis of the semicircular canals is disrupted. Finally, the antytalent locus is involved in late expansion of the ear structure. Analysis of mutations presented here will strengthen our understanding of vertebrate ear morphogenesis and provide novel entry points to its genetic analysis.

McCarthy RA, Sun M, Taylor JC, Smith D. **Polar effects of lithium in the heart of the zebrafish Danio rerio**. Dev Genes Evol 1996;206(2):102-9.
BIOSIS COPYRIGHT: BIOL ABS. The molecular signalling mechanisms that are believed to govern the patterning of the heart early in embryonic development are not well understood. We have investigated the events which occur during patterning of the vertebrate heart by exposing gastrula stage zebrafish embryos to lithium, which is known to affect the phosphoinositol signalling pathway. Treatment of embryos at 50% epiboly (5.25 h after fertilization at 28.5~ C) with 0.3 M LiCl for 5-15 min, results in embryos with defects which range from mild to severe, depending on the length of time the embryos are exposed to lithium. In the heart, defects appear progressively in the inflow tract, the sinus venosus and atrium. By using an antibody that recognizes an atrium-specific isoform of myosin, our results show that lithium treatment at gastrulation specifically affects the atrium and sinus venosus, and has little obvious effect on the ventricle. Defects induced by lithium differ from those induced by retinoic acid (RA) treatment of similarly staged embryos, and suggest that lithium and RA may affect the patterning signals important for establishment of the vertebrate heart by acting on different populations of cells or by influencing different patterning pathways.

Mirkes PE. **Prospects for the development of validated screening tests that measure developmental toxicity potential: view of one skeptic**. Teratology 1996;53(6):334-8.
BIOSIS COPYRIGHT: BIOL ABS. Humans are exposed to a variety of potential developmental toxicants. This fact, combined with the knowledge that human development can be disrupted by environmental agents, has led to the development of methods designed to identify potential developmental toxicants. Currently, the principal method used to screen drugs and chemicals that are potential human developmental toxicants is the segment II study (i.e., a study in which prospective drugs and chemicals are tested in pregnant animals). Because of the cost and time involved in such studies and the pressure to reduce the number of animals used in such testing, alternative methods for developmental toxicity testing have been sought. This has resulted in a number of in vitro tests whose aim is to screen large numbers of agents quickly and inexpensively. Although numerous in vitro tests of developmental toxicity have been developed during the last 15 years, no one system or combination of tests have been validated for the purpose intended. Nonetheless, two systems - the limb bud/CNS micromass, and the chick embryo neural retina cell culture (CERC) - continue to be advanced as viable in vitro developmental toxicology tests. The purpose of this commentary is to evaluate the prospects for the development of an in vitro test system(s) that can screen the universe of drugs and chemicals and reliably identify those that require further study and those that do not. The conclusion of this investigator is that the prospects for validating such in vitro tests are not promising. This conclusion is based

primarily on the lack of basic knowledge regarding the relevance of end points assayed in the micromass and CERC test systems to those end points known or thought to be critical for normal development.

Mizell M, Stegeman JJ, Romig E, Smolowitz R, Schlezinger J, Katayani R, Woodin B, Mortensen M. **Chemically induced cardiovascular defects in developmental stages of vertebrates dose-response and phenotypic comparisons in medaka and zebrafish exposed to aryl hydrocarbon receptor agonists**. Biol Bull 1996;191(2):294-5.
BIOSIS COPYRIGHT: BIOL ABS. RRM meeting abstract oryzias-latipes danio-rerio medaka zebrafish embryo cardiovascular defect 2 3 7 8-tetrachlorodibenzo-p-dioxin embryotoxin tcdd cytochrome p4501a cpy1a development toxicology congenital disease toxicity.

Neuhauss SC, Solnica-Krezel L, Schier AF, Zwartkruis F, Stemple DL, Malicki J, Abdelilah S, Stainier DY, Driever W. **Mutations affecting craniofacial development in zebrafish**. Development 1996;123:357-67.
In a large-scale screen for mutations affecting embryogenesis in zebrafish, we identified 48 mutations in 34 genetic loci specifically affecting craniofacial development. Mutants were analyzed for abnormalities in the cartilaginous head skeleton. Further, the expression of marker genes was studied to investigate potential abnormalities in mutant rhombencephalon, neural crest, and pharyngeal endoderm. The results suggest that the identified mutations affect three distinct aspects of craniofacial development. In one group, mutations affect the overall pattern of the craniofacial skeleton, suggesting that the genes are involved in the specification of these elements. Another large group of mutations affects differentiation and morphogenesis of cartilage, and may provide insight into the genetic control of chondrogenesis. The last group of mutations leads to the abnormal arrangement of skeletal elements and may uncover important tissue-tissue interactions underlying jaw development.

O'Flaherty EJ; Scott W. **Use of toxicokinetics in developmental toxicology**. Handb Dev Toxicol 1997:423-41.
CBAC COPYRIGHT: CHEM ABS A review and discussion with 15 refs. A brief review of classical pharmacokinetic principles and models is presented, together with an introduction to physiol. based models as they are used today and a comparison of the strengths and limitations of the 2 kinds of models. How data sets are fit by models to define and est. dose to the embryo/fetus and how embryo/ fetal dose can be predicted for other exposure conditions and in other species are described. The design and other practical aspects of expts. to generate needed toxicokinetic data are discussed. Finally, applications to practical issues in developmental toxicol. are considered.

Pack M, Solnica-Krezel L, Malicki J, Neuhauss SC, Schier AF, Stemple DL, Driever W, Fishman MC. **Mutations affecting development of zebrafish digestive organs**. Development 1996;123:321-8.
The zebrafish gastrointestinal system matures in a manner akin to higher vertebrates. We describe nine mutations that perturb development of these organs. Normally, by the fourth day postfertilization the digestive organs are formed, the epithelial cells of the intestine are polarized and express digestive enzymes, the hepatocytes secrete bile, and the pancreatic islets and acini generate immunoreactive insulin and carboxypeptidase A, respectively. Seven mutations cause arrest of intestinal epithelial development after formation of the tube but before cell polarization is completed. These perturb different regions of the intestine. Six preferentially affect foregut, and one the hindgut. In one of the

foregut mutations the esophagus does not form. Two mutations cause hepatic degeneration. The pancreas is affected in four mutants, all of which also perturb anterior intestine. The pancreatic exocrine cells are selectively affected in these four mutations. Exocrine precursor cells appear, as identified by GATA-5 expression, but do not differentiate and acini do not form. The pancreatic islets are spared, and endocrine cells mature and synthesize insulin. These gastrointestinal mutations may be informative with regard to patterning and crucial lineage decisions during organogenesis, and may be relevant to diabetes, congenital dysmorphogenesis and disorders of cell proliferation.

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

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

Richard AM, Hunter E3. **Quantitative structure-activity relationships for the developmental toxicity of haloacetic acids in mammalian whole embryo culture**. Teratology 1996;53(6):352-60. BIOSIS COPYRIGHT: BIOL ABS. Developmental toxicity in mouse whole embryo culture assay has been reported for acetic acid (AA) and a series of ten haloacetic acids, including mono-, di-, tri-fluoro (MFA, DFA, TFA), chloro (MCA, DCA, TCA), bromo (MBA, DBA, TBA), and monoiodo (MIA) acetic acids. Benchmark concentrations (BCm), calculated as the lower 95% confidence limit of molar acid concentration producing a 5% increase in embryos with neural tube defects, provided potency estimates for development of quantitative structure-activity relationships (QSARs). The best overall regression was obtained for the ten haloacids (excluding AA) and related log(1/BCm) to the energy of the lowest unoccupied molecular orbital (Elumo) and acid dissociation constant (pKa) with a correlation coefficient of r = 0.97, and a sample size-adjusted r2 = 0.92. This QSAR suggested a common basis for the mechanism of HA activity, which would imply additivity for mixtures of these acids. Examination of QSARs for subsets of the total data set (e.g., monohaloacids) highlighted parameter relationships

embedded in the total QSAR, helping to unravel the separate contributions of Elumo and pKa to the overall potency. The relevance of these parameters is discussed in terms of postulated mechanisms of developmental toxicity involving changes in intercellular pH and redox metabolism. The whole embryo assay results pertain to direct embryo exposure and toxicity without the confounding influence of maternal factors. The resulting QSAR model offers possible insight into the mechanism of embryo toxicity that will hopefully contribute to understanding of the more complex, in vivo teratogenicity problem.

Schier AF, Joyner AL, Lehmann R, Talbot WS. **From screens to genes: prospects for insertional mutagenesis in zebrafish [comment]**. Genes Dev 1996;10(24):3077-80.

Schier AF, Neuhauss SC, Harvey M, Malicki J, Solnica-Krezel L, Stainier DY, Zwartkruis F, Abdelilah S, Stemple DL, Rangini Z, et al. **Mutations affecting the development of the embryonic zebrafish brain**. Development 1996;123:165-78.
In a large scale mutagenesis screen for embryonic mutants in zebrafish, we have identified 63 mutations in 24 loci affecting the morphogenesis of the zebrafish brain. The expression of marker genes and the integrity of the axonal scaffold have been studied to investigate abnormalities in regionalization, neurogenesis and axonogenesis in the brain. Mutants can be broadly classified into two groups, one affecting regionalization along the anterior-posterior or dorsal-ventral axis, and the other affecting general features of brain morphology. The first group includes one locus that is required to generate the anlage of the midbrain-hindbrain boundary region at the beginning of somitogenesis. Four loci were identified that affect dorsal-ventral patterning of the brain, including the previously described cyclops locus. Mutant embryos of this class show a reduction of ventral neuroectodermal structures and variable fusion of the eyes. The second group includes a large class of mutations affecting the formation of brain ventricles. Analysis of this class reveals the requirement of a functional cardiovascular system for ventricle enlargement during embryogenesis. Mutations in one locus lead to the formation of supernumerary primary neurons, a phenotype reminiscent of neurogenic mutants in Drosophila. Other mutant phenotypes described here range from abnormalities in the fasciculation and outgrowth of axons to defects in the diameter of the neural tube. The identified loci establish the genetic foundation for a further analysis of the development of the zebrafish embryonic brain.

Schmahl HJ, Dencker L, Plum C, Chahoud I, Nau H. **Stereoselective distribution of the teratogenic thalidomide analogue EM12 in the early embryo of marmoset monkey, Wistar rat and NMRI mouse**. Arch Toxicol 1996;70(11):749-56.
BIOSIS COPYRIGHT: BIOL ABS. Thalidomide administration during early gestation results in specific and dramatic limb defects in primates, but not in laboratory rodents such as the rat and mouse. The thalidomide analogue EM 12 (2-(2, 6-dioxopiperidine-3-yl)-phthalimidine) was used in the present study because this compound is metabolically more stable and teratogenically more potent than thalidomide in the monkey. We have administered the pure enantiomers, since we have previously shown that S-EM12 proved to be much more teratogenic in the monkey than R-EM12. In maternal plasma, placenta and embryo of the pregnant marmoset monkey (Callithrix jacchus) and Wistar rat, the concentrations were investigated of the enantiomers and their metabolites after administration of R- and S-EM12. With whole body autoradiography the distribution in the embryo, including the target tissue,

the embryonic limb bud was examined in the NMRI mouse and marmoset monkey. Our investigations showed that both the R- and the S-enantiomers were transferred to the embryo during organogenesis (monkey, gestation day (GD) 61; rat, GD 12; mouse, GD 10). The gestation period chosen was toward the end of the thalidomide-sensitive stage, but yielded sufficient gestational material for analysis. Considerable amounts of the enantiomers were produced via racemization of the administered pure enantiomers and were present in maternal plasma as well as in placenta and embryo. In the monkey, the racemization were stereoselective: the S-enantiomer was eliminated more slowly in the monkey than the R-enantiomer, possibly because of stereospecific binding and metabolism. In the plasma and embryo of both rat and monkey, the metabolites were detected in considerably lower concentrations than EM12, emphasizing the importance of the parent drug in regard to the teratogenic effect. The whole-body autoradiography in marmoset and mouse showed high radioactivity in the embryonic CNS, the branchial apparatus and in the limb buds. The S-enantiomer of EM12 was more strongly concentrated than the R-enantiomer in these areas. In the limb buds, the highest concentrations of radioactivity were observed in the periphery, sometimes at the very tip of the buds. Accumulation of radioactivity in limb buds and neural epithelium relative to other areas of the embryo was much more pronounced in the monkey than in the mouse. Future studies must demonstrate if this accumulation has implications for the mechanism of thalidomide teratogenesis in primate species.

Solnica-Krezel L, Stemple DL, Mountcastle-Shah E, Rangini Z, Neuhauss SC, Malicki J, Schier AF, Stainier DY, Zwartkruis F, Abdelilah S, et al. **Mutations affecting cell fates and cellular rearrangements during gastrulation in zebrafish**. Development 1996;123:67-80.
One of the major challenges of developmental biology is understanding the inductive and morphogenetic processes that shape the vertebrate embryo. In a large-scale genetic screen for zygotic effect, embryonic lethal mutations in zebrafish we have identified 25 mutations that affect specification of cell fates and/or cellular rearrangements during gastrulation. These mutations define at least 14 complementation groups, four of which correspond to previously identified genes. Phenotypic analysis of the ten novel loci revealed three groups of mutations causing distinct effects on cell fates in the gastrula. One group comprises mutations that lead to deficiencies in dorsal mesodermal fates and affect central nervous system patterning. Mutations from the second group affect formation of ventroposterior embryonic structures. We suggest that mutations in these two groups identify genes necessary for the formation, maintenance or function of the dorsal organizer and the ventral signaling pathway, respectively. Mutations in the third group affect primarily cellular rearrangements during gastrulation and have complex effects on cell fates in the embryo. This group, and to some extent mutations from the first two groups, affect the major morphogenetic processes, epiboly, convergence and extension, and tail morphogenesis. These mutations provide an approach to understanding the genetic control of gastrulation in vertebrates.

Sordino P, Duboule D, Kondo T. **Zebrafish Hoxa and Evx-2 genes: cloning, developmental expression and implications for the functional evolution of posterior Hox genes**. Mech Dev 1996;59 (2):165-75.
CBAC COPYRIGHT: CHEM ABS Vertebrate Hox genes are required for the establishment of regional identities along body axes. This gene family is strongly conserved among vertebrates, even in bony fish which display less complex ranges of axial morphologies. We have analyzed the structural organization

and expression of Abd-B related zebrafish HoxA cluster genes (Hoxa-9, Hoxa-10, Hoxa-11 and Hoxa-13) as well as of Evx-2, a gene closely linked to the HoxD complex. We show that the genomic organization of Hoxa genes in fish resembles that of tetrapods albeit intergenic distances are shorter. During development of the fish trunk, Hoxa genes are coordinately expressed, whereas in pectoral fins, they display transcript domains similar to those obsd. in developing tetrapod limbs. Likewise, the Evx-2 gene seems to respond to both Hox- and Evx-types of regulation. During fin development, this latter gene is expressed as the neighboring Hox genes, in contrast to its expression in the central nervous system which does not comply with colinearity and extends up to anterior parts of the brain. These results are discussed in the context of the functional evolution of Hoxa vs. Hoxd genes and their different roles in building up paired appendages.

Stainier DY, Fouquet B, Chen JN, Warren KS, Weinstein BM, Meiler SE, Mohideen MA, Neuhauss SC, Solnica-Krezel L, Schier AF, et al. **Mutations affecting the formation and function of the cardiovascular system in the zebrafish embryo**. Development 1996;123:285-92.
As part of a large-scale mutagenesis screen of the zebrafish genome, we have identified 58 mutations that affect the formation and function of the cardiovascular system. The cardiovascular system is particularly amenable for screening in the transparent zebrafish embryo because the heart and blood vessels are prominent and their function easily examined. We have classified the mutations affecting the heart into those that affect primarily either morphogenesis or function. Nine mutations clearly disrupt the formation of the heart. cloche deletes the endocardium. In cloche mutants, the myocardial layer forms in the absence of the endocardium but is dysmorphic and exhibits a weak contractility. Two loci, miles apart and bonnie and clyde, play a critical role in the fusion of the bilateral tubular primordia. Three mutations lead to an abnormally large heart and one to the formation of a diminutive, dysmorphic heart. We have found no mutation that deletes the myocardial cells altogether, but one, pandora, appears to eliminate the ventricle selectively. Seven mutations interfere with vascular integrity, as indicated by hemorrhage at particular sites. In terms of cardiac function, one large group exhibits a weak beat. In this group, five loci affect both chambers and seven a specific chamber (the atrium or ventricle). For example, the weak atrium mutation exhibits an atrium that becomes silent but has a normally beating ventricle. Seven mutations affect the rhythm of the heart causing, for example, a slow rate, a fibrillating pattern or an apparent block to conduction. In several other mutants, regurgitation of blood flow from ventricle to atrium is the most prominent abnormality, due either to the absence of valves or to poor coordination between the chambers with regard to the timing of contraction. The mutations identified in this screen point to discrete and critical steps in the formation and function of the heart and vasculature.

Tyler CR, Van d Eerden B, Jobling S, Panter G, Sumpter JP. **Measurement of vitellogenin, a biomarker for exposure to oestrogenic chemicals, in a wide variety of cyprinid fish**. J Comp Physiol [B] 1996; 166(7):418-26.
BIOSIS COPYRIGHT: BIOL ABS. There is increasing concern about man-made chemicals in the aquatic environment that mimic oestrogens because they may disrupt reproductive function. Vitellogenin, a precursor of egg-yolk in fish and other oviparous animals, may be used as a biomarker for "oestrogen" exposure. This study investigated the use of a radioimmunoassay developed to carp (Cyprinus carpio) vitellogenin to measure vitellogenin in other species of fish, especially cyprinids that would be of value for field and laboratory studies on oestrogenic xenobiotics. Of the nine families of

fish studied, only vitellogenin from cyprinids (to which the carp belongs) showed good cross-reactivity in the carp vitellogenin radioimmunoassay. Vitellogenin from cyprinids native to Europe that cross reacted in the carp vitellogenin radioimmunoassay included: bream (Abramis brama), roach (Rutilus rutilus), rudd (Scardinius erythropthalmus), gudgeon (Gobio gobio) and minnow (Phoxinus phoxinus). Vitellogenin from cyprinids used widely in ecotoxicology that cross reacted in the carp vitellogenin radioimmunoassay included: fathead minnow (Pimephales promelas), zebrafish (Brachydanio rerio) and goldfish (Carassius auratus). In the cyprinids studied, the concentrations of vitellogenin in mature females were between a few hundred and a thousand microgram per millilitre. Concentrations of plasma vitellogenin in immature females were always greater than 200 ngathead minnow) plasma vitellogenin concentrations were less than 20 ng that the structure of vitellogenin is highly conserved within the cyprinid family and that the carp vitellogenin radioimmunoassay may be used to measure the concentrations of vitellogenin in plasma from a wide variety of cyprinids.

Weinstein BM, Schier AF, Abdelilah S, Malicki J, Solnica-Krezel L, Stemple DL, Stainier DY, Zwartkruis F, Driever W, Fishman MC. **Hematopoietic mutations in the zebrafish**. Development 1996;123:303-9.
We have identified mutations that perturb the formation or differentiation of the first embryonic blood cells in the zebrafish embryo. These 'primitive' red blood cells originate in the intermediate cell mass of the trunk, a derivative of the dorsal lateral plate mesoderm. By transfusion of blood between embryos we demonstrate that this cohort of cells provides the embryo with all, or nearly all, of its blood cells until at least day 5 postfertilization. Larval lethal mutations generated by ENU mutagenesis affect different steps in the development of these cells. Some cause defects in precursor generation, others defects in differentiation, and others an increase in cellular photosensitivity.

Whitfield TT, Granato M, Van Eeden FJ, Schach U, Brand M, Furutani-Seiki M, Haffter P, Hammerschmidt M, Heisenberg CP, Jiang YJ, et al. **Mutations affecting development of the zebrafish inner ear and lateral line.** Development 1996;123:241-54.
Mutations giving rise to anatomical defects in the inner ear have been isolated in a large scale screen for mutations causing visible abnormalities in the zebrafish embryo (Haffter, P., Granato, M., Brand, M. et al. (1996) Development 123, 1-36). 58 mutants have been classified as having a primary ear phenotype.

Zeevalk GD, Nicklas WJ. **Attenuation of excitotoxic cell swelling and GABA release by the GABA transport inhibitor SKF 89976A**. Mol Chem Neuropathol 1996;29(1):27-36.
Acute excitotoxicity in the chick retina is characterized by cellular swelling and the subsequent selective release of GABA. In order to understand the source of GABA release, embryonic day 15 retina were incubated with 1 mM glutamate for 30 min in the presence or absence of the GABA transport inhibitor SKF 89976A (1-100 microM). SKF 89976A dose-dependently attentuated glutamate-induced GABA release (IC50, 39 microM). Histological examination of retina showed that SKF 89976A greatly reduced cellular swelling caused by glutamate exposure. Interaction of SKF 89976A with glutamate receptors was ruled out as a possible reason for protection vs acute glutamate excitotoxicity, since SKF 89976A had no effect on glutamate receptor-induced 22Na+ influx. In contrast, the NMDA antagonist, MK-801, significantly blocked glutamate-evoked 22NA+ uptake. These studies indicate that reversal of the GABA transporter contributes to the bulk of GABA release during acute excitotoxicity in retina. Further, a net effect of the presence of SKF 89976A during glutamate exposure is reduction in cellular

swelling. It is not clear at present if attenuation of swelling is mediated specifically by an interaction with the GABA transporter or by a nonspecific or indirect effect of SKF 89976A.

MISCELLANEOUS

Campbell CA, Teschke K, Bart J, Quintana PJ, Hertzman C. **Pharmacokinetic model of dioxin and furan levels in adipose tissue from sawmill work involving chlorophenate fungicides**. Chemosphere 1996; 33(12):2373-81.
BIOSIS COPYRIGHT: BIOL ABS. Sawmill workers in British Columbia (B.C.), Canada, have been exposed to chlorophenate fungicides which are known to be contaminated with polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Due to concern about the potential of these workers to have significant body burdens of PCDD/Fs, an the absence of measurements in these workers, a single-compartment pharmacokinetic model was developed to estimate the concentration of PCDD/Fs in the fat tissue of the sawmill workers. Data from a large cohort of B.C. sawmill workers and literature-based data on chlorophenate exposures and PCDD/F concentrations in chlorophenates were used in Monte Carlo simulations to predict a PCDD/F body burden distribution. The median concentrations of HxCDF and HpCDF predicted using the model for the B.C. sawmill worker population exceeded the range measured in unexposed populations. PeCDF and OCDF concentrations exceeded the range measured in unexposed populations at the 70th percentile of the model-predicted distribution, and PeCDD at the 90th percentile. The primary limitation of the model was the scarcity of input data about actual dermal and inhalation exposures to chlorophenates.

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

Crump KS. **The linearized multistage model and the future of quantitative risk assessment**. Hum Exp Toxicol 1996;15(10):787-98.
The linearized multistage (LMS) model has for over 15 years been the default dose-response model used by the U.S. Environmental Protection Agency (USEPA) and other federal and state regulatory agencies in the United States for calculating quantitative estimates of low-dose carcinogenic risks from animal data. The LMS model is in essence a flexible statistical model that can describe both linear and non-

linear dose-response patterns, and that produces an upper confidence bound on the linear low-dose slope of the dose-response curve. Unlike its namesake, the Armitage-Doll multistage model, the parameters of the LMS do not correspond to actual physiological phenomena. Thus the LMS is 'biological' only to the extent that the true biological dose response is linear at low dose and that low-dose slope is reflected in the experimental data. If the true dose response is non-linear the LMS upper bound may overestimate the true risk by many orders of magnitude. However, competing low-dose extrapolation models, including those derived from 'biologically-based models' that are capable of incorporating additional biological information, have not shown evidence to date of being able to produce quantitative estimates of low-dose risks that are any more accurate than those obtained from the LMS model. Further, even if these attempts were successful, the extent to which more accurate estimates of low-dose risks in a test animal species would translate into improved estimates of human risk is questionable. Thus, it does not appear possible at present to develop a quantitative approach that would be generally applicable and that would offer significant improvements upon the crude bounding estimates of the type provided by the LMS model. Draft USEPA guidelines for cancer risk assessment incorporate an approach similar to the LMS for carcinogens having a linear mode of action. However, under these guidelines quantitative estimates of low-dose risks would not be developed for carcinogens having a non-linear mode of action; instead dose-response modelling would be used in the experimental range to calculate an LED10* (a statistical lower bound on the dose corresponding to a 10% increase in risk), and safety factors would be applied to the LED10* to determine acceptable exposure levels for humans. This approach is very similar to the one presently used by USEPA for non-carcinogens. Rather than using one approach for carcinogens believed to have a linear mode of action and a different approach for all other health effects, it is suggested herein that it would be more appropriate to use an approach conceptually similar to the 'LED10*-safety factor' approach for all health effects, and not to routinely develop quantitative risk estimates from animal data.

D'Ambrosio G, Lioi MB, Massa R, Scarfi MR, Zeni O. **Genotoxic effects of amplitude-modulated microwaves on human lymphocytes exposed In vitro under controlled conditions**. Electro Magnetobiol 1995;14(3):157-64.
Human blood samples were exposed to continuous wave (CW) and 50 hertz (Hz) amplitude modulated (AM) radiation. Lymphocytes were prepared from blood samples from 23 healthy donors, 23 to 95 years old. For four donors, lymphocytes were prepared from blood samples obtained on more than one occasion. Two exposure conditions were used. In the one, samples were exposed to 9 gigahertz (GHz) CW microwaves for 10 minutes. In the second, samples were exposed to 9GHz microwaves AM modulated at 50Hz for 10 minutes. Control cultures were also prepared for each subject. The cytokinesis block micronucleus method was used to evaluate possible genotoxic effects. The findings did not produce evidence of genotoxic effects in cases of CW irradiation. A significant increase in micronuclei was found following the AM microwave exposure. No influence of donor age was seen. The authors conclude that these results confirm the importance of extremely low frequency amplitude modulated microwaves.

Escoubas P, Palma MF, Nakajima T. **A microinjection technique using Drosophila melanogaster for bioassay-guided isolation of neurotoxins in arthropod venoms**. Toxicon 1995;33(12):1549-55.
Modern analytical techniques permit isolation and structural determination of neurotoxins at the

picomole level. However, bioassay-guided fractionation of the sample often relies on simple injection assays using insects, vertebrates or crustaceans of a fairly large size, thus consuming quite a large amount of the samples being investigated. In order to investigate samples of very small size, we have devised an insect microinjection method using glass micropipettes and Drosophila melanogaster adults as test insects. The validity of the method was tested with a series of six buthoid scorpion venoms (Androctonus australis, Buthotus judaicus, Buthus tamulus, Centruroides sculpturatus, Leiurus quinquestriatus hebraeus, Tityus serrulatus) and one chactoid scorpion (Scorpio maurus palmatus) as standards. The LD50S of the venoms were determined using both the microinjection method and a classical injection assay with crickets (Gryllus bimaculatus) as test insects. Results demonstrated that the new method can successfully be applied to the study of insect neurotoxic activity in arthropod venoms. The Gryllus:Drosophila ratio in amount of sample utilized is 100. However, for all Buthoid venoms tested, except L. quinquestriatus, Drosophila showed less sensitivity, thus reducing the gain by a factor of 2-10. Drosophila were several times more sensitive to the only chactoid venom tested. These results clearly demonstrate the advantage of using this microtechnique, when limited amounts of material are available for both chemical and biological work.

Firsov AA, Ruble M, Gilbert D, Savarino D, Manzano B, Medeiros AA, Zinner SH. **Net effect of inoculum size on antimicrobial action of ampicillin-sulbactam: studies using an in vitro dynamic model**. Antimicrob Agents Chemother 1997;41(1):7-12.
CBAC COPYRIGHT: CHEM ABS To examine the predictable effect of inoculum size on the kinetics of the antimicrobial action of ampicillin-sulbactam, five TEM-1 beta-lactamase-producing Escherichia coli strains were studied in an in vitro dynamic model at two different initial inocula (N0s). All bacteria were exposed to ampicillin-sulbactam in a simulated system reflecting the pharmacokinetic profiles in human tissue after the administration of a single i.v. dose of ampicillin (2 g) plus sulbactam (1 g). Each strain was studied at low (4.0 to 5.2 log CFU/mL) and high (5.0 to 7.1 log CFU/mL) N0s. Despite pronounced differences in susceptibilities, the patterns of the killing curves obsd. with a given strain at different N0s were similar. As expected, viable bacterial counts increased with inoculum size. Striking visual contrasts in the resp. curves for each organism were reflected by the area under the bacterial count-time curve (AUBC) but not by the difference between the N0 and the lowest bacterial counts (Nmin) at the nadir of the killing curve: the N0-assocd. changes in the AUBC on av. were 75%, vs. 2.5% for log N0-log Nmin. To examine qual. differences in antimicrobial effects at different N0s (i.e., the net effect of the inoculum), the difference in the high and low N0s was subtracted from each point on the killing curves obtained at the lower N0. Moreover, by using adjusted data, the AUBC values were similar at the two inocula, although slight (av., 11%) but systematic increases in the AUBC occurred at high N0s. Thus, there was only a weak net effect of inoculum size on the antibacterial effect of ampicillin-sulbactam. Due to similar slopes of the AUBC-log N0 plots, the antibacterial action at different N0s may be easily predicted by an approx. equation; the predicted AUBCs were unbiased and well correlated with the obsd. AUBCs (r = 0.997). Compiled data obtained with normalized AUBCs for different strains at different N0s yielded a pos. correlation (r = 0.963) between the N0-normalized AUBC and the MIC of ampicillin-sulbactam. The adjustment and normalization procedure described might be a useful tool for revealing the net effect of the inoculum and to predict the inoculum effect if there are no qual. differences in antimicrobial action at different inocula.

Fischer R, Rehn B, Bruch J. **Dust specific effects on the macrophage pneumocyte type II cell system comparing in vitro and in vivo data**. Exp Toxicol Pathol 1996;48(6):520-2.
BIOSIS COPYRIGHT: BIOL ABS. RRM RESEARCH ARTICLE WISTAR RAT FEMALE MACROPHAGE PNEUMOCYTE TYPE II CELL SYSTEM DUST SPECIFIC EFFECT ALVEOLAR MACROPHAGE TOXICOLOGY PATHOGENIC PATHWAY TOXICODYNAMICS BLOOD AND LYMPHATICS RESPIRATORY SYSTEM.

Girardot JM, Girardot MN. **Amide cross-linking: an alternative to glutaraldehyde fixation**. J Heart Valve Dis 1996;5(5):518-25.
BACKGROUND AND AIMS OF THE STUDY: A new fixation method for bioprosthetic tissues is being developed, which does not utilize the standard glutaraldehyde treatment. This method, referred to as Ultifix, uses a coupler and a coupling enhancer with or without one or more coupling agents. It fixes the tissue by linking the amine and the carboxyl moieties through amide bonds either directly, or indirectly when coupling agents form bridges. The amide bonds thus formed are more stable than the Schiff-base bonds formed by glutaraldehyde. All compounds used during the fixation process and their by-products are water-soluble, and are easily removed by washing. In addition, the by-products are not toxic, as opposed to glutaraldehyde, which induces toxic reactions after implantation. The tests described in the manuscript were specifically aimed at evaluating the cross-linking efficacy of the process on heart valve tissues, as well as their resistance to calcification in the rat model. METHODS: Porcine aortic roots and porcine pericardium were fixed using the coupling agents 1,6-hexane diamine (DIA) and suberic acid (SUA) in the presence of the coupler 1-ethyl-3(-3 dimethyl.

Guth DJ, Raymond TS. **A database designed to support dose-response analysis and risk assessment**. Toxicology 1996;114(1):81-90.
Risk assessment for various human exposures depends on evaluation of existing toxicological literature from a variety of sources. Risk assessors may have limited resources for obtaining raw data, performing additional analyses and initiating new laboratory or epidemiological studies. These constraints must be balanced against a need to improve scientific credibility by developing improved statistical and analytical methods that optimize the use of the available information. A database is described that was designed specifically to support emerging analytical approaches for dose-response assessment, while accommodating the diverse nature of published literature. The database allows entry of exposure and response information in a relational multi-table design, with closely controlled standard fields for recording values and free-text fields for describing unique aspects of a study. To include data needed for current as well as proposed methods, multiple fields were created for different data types and for exposure characterization. The database structure allows rapid access to, and versatile use of, toxicological data for dose-response analyses.

Hou SY E, Flynn GL. **Influences of 1-dodecylazacycloheptan-2-one on permeation of membranes by weak electrolytes. 1. Theoretical analysis of weak electrolyte diffusion through membranes and studies involving silicone rubber membranes**. J Pharm Sci 1997;86(1):85-91.
CBAC COPYRIGHT: CHEM ABS The pH dependency of permeation of weak electrolytes allows inferences to be made about the barrier characteristics of membranes. The influences of enhancers on pH-permeation profiles promise further mechanistic enlightenment. To explore issues of weak electrolyte mass transfer, a steady-state math. model for a hydrophobic membrane with aq. pores

existing in series with aq. phases, presently a popular depiction of the skin and other biol. barriers, has been developed. The case in which there are no pores is then considered theor. and in studies involving the mass transfer of benzoic acid across silicone rubber membranes. Specifically, the flux of [14C] benzoic acid across Silastic sheeting as a function of pH was investigated. This isotropic membrane's behavior conformed to expectations drawn from the model in that the un-ionized species penetrated in proportion to benzoic acid's prevailing state of ionization, the membrane being all but impenetrable to the benzoate anion. The enhancer, 1-dodecylazacycloheptan-2-one (Azone), was then applied to the membrane in emulsions of increasing concn. There were 2 important consequences of such application. First, the un-ionized species of benzoic acid partitioned into the emulsion droplets, lowering the activity of the permeant in the emulsion's continuous phase. Second, Azone was imbibed to a degree into the polymeric membrane, significantly altering the permeability of the silicone rubber of which it is composed. The former influence had to be carefully factored out in order to delineate Azone's intrinsic enhancing effects on the membrane. The silicone rubber membrane system served well as a model for study of the enhancing effects of Azone on a wholly hydrophobic barrier, establishing a basis for the anal. of the actions of enhancers such as Azone on more complex, multiphasic biol. barriers.

Lovich MA, Edelman ER. **Computational simulations of local vascular heparin deposition and distribution**. Am J Physiol 1996;271(5):H2014-H2024.
CBAC COPYRIGHT: CHEM ABS Local vascular drug delivery systems provide elevated concns. in target arterial tissues while minimizing systemic side effects; however, definition of their precise pharmacokinetics remains elusive. The std. labeled tracer assays used in exptl. vascular pharmacokinetic studies of these systems are limited because they quantify the arterial av. drug concn. as opposed to transmural concn. profiles, require many animal expts. to elucidate the time-varying deposition, and track label rather than intact biol. active drug. In this study, computational simulations of drug deposition and distribution in vascular tissues after release from these systems have provided 2 important insights. First, simulations of arteries that were uniformly loaded with heparin predicted that most of the drug is cleared in <1 h, illustrating the need for sustained modes of delivery. Second, some of the limitations of labeled tracers can be overcome by combining exptl. data with simulations that provide high spatial resoln. This enabled the authors to describe the kinetics of the deposited drug and distinguish sol. from reversibly bound and internalized drug within cells. The latter can help differentiate biol. viable drug from its committed inactive form or metabolites. These points have been illustrated through simulations of a novel endovascular hydrogel heparin-delivery system that has been applied to the porcine coronary artery. The basic models used in these simulations are generalized, and with the appropriate boundary conditions, binding and distribution consts. can be used to study the phys. interactions between any compd. and tissue.

Lu G, Lang P. **[The structure and toxicity relationship study for nitroaromatics to Scenedesmus obliquus]**. Huanjing Kexue 1996;17(2):35-6, 59. (Chi)
CBAC COPYRIGHT: CHEM ABS The energy of low unoccupied MO (ELUMO), the energy of high occupied MO (EHOMO), the difference of formation heat DELTA(DELTAHf), the dipolar moment (mu), and the net elec. charge (Q-NO2) of 18 nitroarom. compds. were calcd. by the quantum chem. method MNDO. The quant. structure-activity relationships (QSAR) were studied in terms of the five quantum chem. descriptors for the acute toxicity of the nitroaroms. to Scenedesmus obliquus. An

equation to relate the half effective suppression concn. of a nitroarom. compd. with the DELTA (DELTAHf), mu, and EHOMO was obtained by regression anal., and the toxicity of each of the nitroarom. compds. was calcd.

Luis Kemper E, Joseda Silva M, Arruda P. **Effect of microprojectile bombardment parameters and osmotic treatment on particle penetration and tissue damage in transiently transformed cultured immature maize (Zea mays L.) embryos**. Plant Sci 1996;121(1):85-93.
CBAC COPYRIGHT: CHEM ABS Immature maize (Zea mays L.) embryos of the tropical inbred line Cat-100-6, plated in callus induction medium for short periods, were used to study the effect of microprojectile bombardment parameters and osmotic treatment on the transformation of embryogenic competent cells. Somatic embryogenesis in this material arose from sub-epidermal cell clusters located at the third or deeper cell layer. Expts. were carried out focusing on the transformation of these cell clusters following microprojectile bombardment. Phys. conditions such as particle delivering method, particle size, helium pressure and target distance were analyzed in factorial expts. The best condition which permitted the transformation of embryogenic cell clusters at relatively high frequency was found to cause deleterious tissue damage because of the bombardment at high pressure and short target distance. Tissue damage as successfully prevented by the addn. of mannitol in the culture medium before and after bombardment.

Menudier A, Rougier FP, Bosgiraud C. **Comparative virulence between different strains of Listeria in zebrafish (Brachydanio rerio) and mice**. Pathol Biol 1996;44(9):783-9.
Listeriosis is a disease found in most animal species but is relatively uncommon in fish. We studied the relationship between Listeria and zebrafish by injecting Brachydanio rerio intraperitoneally with different Listeria strains having pathological or food-stuff origins. We then compared these results with those obtained in Swiss mice. Experimental Listeriosis in Zebrafish differs greatly from that observed in mice. The 50% lethal dose (LD50) previously determined was much higher than that observed in mice. In fish, a good correlation exists between infection found in renal tissue, an important lymphoid organ and that present in whole fish ($p < 0.001$). Infection kinetics showed that, in contrast with mice, L. monocytogenes was unable to multiply in fish. Differential blood counts showed the development of an immune response in fish. The difference in the expression of Listeria virulence between Zebrafish and mice was also seen in their reactions to different wild strains inoculate i.p. Strains belonging the innocua, ivanovii, seeligeri and welshimeri were weakly or not virulent in mice but virulent in fish. Nevertheless, as in mice, differences in virulence existed between strains of L. monocytogenes belonging to serovars 4b, 1/2a, 1/2b and 1/2c.

Nihlen A, Lof A, Johanson G. **Liquid/air partition coefficients of methyl and ethyl T-butyl ethers, T-amyl methyl ether, and T-butyl alcohol**. J Expo Anal Environ Epidemiol 1995;5(4):573-82.
Partition coefficients are essential to a description of the uptake and distribution of volatile substances in humans and in the development of physiologically based pharmacokinetic models. Liquid/air partition coefficients (lambda) of three ethers, methyl t-butyl ether (MTBE), ethyl t-butyl ether (ETBE), and t-amyl methyl ether (TAME) were determined in vitro by head space-gas chromatography. These ethers, and especially MTBE, are used in unleaded gasoline to enhance the oxygen and octane content, and to reduce the output of carbon monoxide during combustion. Partition coefficients of t-butyl alcohol (TBA), a metabolite of MTBE, were determined also. The liquids tested were fresh human blood, water

(physiological saline), and olive oil. The (lambda)blood/air values were: 17.7 (95% confidence interval 17.0-18.4) for MTBE; 11.7 (11.3-12.1) for ETBE; and 17.9 (17.3-18.5) for TAME. Corresponding (lambda)water/air values were 15.2 (14.9-15.5), 8.39 (8.19-8.59), and 11.9 (11.7-12.1). The ethers have a higher affinity for oil, the values for (lambda)oil/air being 120 (114-125), 190 (183-197), and 337 (320-354), respectively. As expected, the (lambda)blood/air and (lambda)water/air for TBA were much higher than for the ethers, 462 (440-484) and 603 (590-617), respectively. The (lambda)oil/air was 168 (161-174) for TBA. The interindividual variability of the (lambda)blood/air (10 subjects) was calculated as the coefficient of variation, and estimated as: 14% for MTBE, 20% for ETBE, 20% for TAME, and 30% for TBA. No significant difference was seen in the (lambda)blood/air between the sexes.

Pai SM, Fettner SH, Hajian G, Cayen MN, Batra VK. **Characterization of AUCs from sparsely sampled populations in toxicology studies**. Pharm Res 1996;13(9):1283-90.
PURPOSE: The objective of this work was to develop and validate blood sampling schemes for accurate AUC determination from a few samples (sparse sampling). This will enable AUC determination directly in toxicology studies, without the need to utilize a large number of animals. METHODS: Sparse sampling schemes were developed using plasma concentration-time (Cp-t) data in rats from toxicokinetic (TK) studies with the antiepileptic felbamate (F) and the antihistamine loratadine (L); Cp-t data at 13-16 time-points (N = 4 or 5 rats/time-point) were available for F, L and its active circulating metabolite descarboethoxyloratadine (DCL). AUCs were determined using the full profile and from 5 investigator designated time-points termed critical time-points. Using the bootstrap (re-sampling) technique, 1000 AUCs were computed by sampling (N = 2 rats/point, with replacement) from the 4 or 5 rats at each critical point. The data were subsequently modeled using PCNONLIN, and the parameters (ka, ke, and Vd) were perturbed by different degrees to simulate pharmacokinetic (PK) changes that may occur during a toxicology study due to enzyme induction/inhibition, etc. Finally Monte Carlo simulations were performed with random noise (10 to 40%) applied to Cp-t and/or PK parameters to examine its impact on AUCs from sparse sampling. RESULTS: The 5 time-points with 2 rats/point accurately and precisely estimated the AUC for F, L and DCL; the deviation from the full profile was approximately 10%, with a precision (%CV) of approximately 15%. Further, altered kinetics and random noise had minimal impact on AUCs from sparse sampling. CONCLUSIONS: Sparse sampling can accurately estimate AUCs and can be implemented in rodent toxicology studies to significantly reduce the number of animals for TK evaluations. The same principle is applicable to sparse sampling designs in other species used in safety assessments.

Paine AJ. **Validity and reliability of in vitro systems in safety evaluation**. Environ Toxicol Pharmacol 1996;2(2-3):207-12.
BIOSIS COPYRIGHT: BIOL ABS. In vitro data could make an important contribution to the application of the proposed scheme for the subdivision of the usual 10-fold safety factors (used in risk assessment for inter-species and inter-individual differences) into two separate aspects of toxicokinetics and toxicodynamics. Whereas toxicokinetics (or delivery of the chemical to its site of action via the general circulation) is amenable to direct in vivo measurement, toxicodynamics (or the assessment of the sensitivity of the target tissue to the presence of the chemical) is open to in vitro investigation. Human risk assessment requires human data to be able to replace any of the default safety (or uncertainty) factors (Renwick, 1993). Because human tissues are of limited availability, it is likely that the main

quantitative contribution of in vitro data will be to allow chemical specific inter-species differences in toxicodynamics to replace the proposed default value. Although in vitro data from human tissues could be used to define human variability in target organ sensitivity (toxicodynamics) this would require a large number of specimens and the variability detected in vitro should be representative of that present in vivo.

Pang SZ, Deboer DL, Wan Y, Ye G, Layton JG, Neher MK, Armstrong CL, Fry JE, Hinchee MA, Fromm ME. **An improved green fluorescent protein gene as a vital marker in plants**. Plant Physiol 1996;112(3):893-900.
A synthetic green fluorescent protein (GFP) gene (pgfp) was constructed to improve GFP expression in plants. Corn and tobacco protoplast transient assays showed that pgfp gave about 20-fold brighter fluorescence than the wild-type gene (gfp). Replacement of the serine at position 65 with a threonine (S65Tpgfp) or a cysteine (S65Cpgfp) yielded 100- to 120-fold brighter fluorescence than wild-type gfp upon excitation with 490-nm light. Incorporation of a plant intron into the coding region yielded an additional 1.4-fold improvement, for a cumulative improvement of about 150-fold in fluorescence at 490-nm excitation. Various versions of pgfp were also stably introduced into corn, wheat, tobacco, and Arabidopsis plants. Bright-green fluorescence was observed with a fluorescence microscope in virtually all examined tissues of transgenic monocots and dicots. In the case of Arabidopsis, expression of the pgfp gene under the enhanced 355 promoter of the cauliflower mosaic virus produced green fluorescence that was readily detectable by eye using a hand-held, long-wave ultraviolet lamp and/or a black-light source.

Paolini M, Pozzetti L, Pedulli GF, Cipollone M, Mesirca R, Cantelli-Forti G. **Paramagnetic resonance i detecting carcinogenic risk from cytochrome P450 overexpression**. J Investig Med 1996;44(8):470-3.
BACKGROUND: Despite the increasing interest in the role of oxygen radicals on human degenerative disorders including cancer, oxidative stress status is not yet measurable in vivo, largely precluding clinical application. Limited semi-quantitative assays of damage to broad classes of biomolecules such as lipids, proteins, and DNA are currently available. The detection of radicals in humans by a whole-body electron paramagnetic resonance (EPR) technique has not yet been developed, although this possibility has long fascinated free radical investigators. METHODS: While the EPR spin trapping procedure can be used to detect carbon centered or hydroxyl radical in human tissues, the most common spin traps are much less useful for capturing the superoxide anion (O2). To overcome these limitations, we propose a whole-body-harvest approach that utilizes a highly lipophilic spin scavenger that when injected in the animal is capable of trapping the O2 generated in vivo throughout the body with formation of a stable nitroxide measurable by EPR in the urine. A process known to generate the O2 is the induction of certain cytochrome P450 (CYP) isozymes by drugs or environmental pollutants. RESULTS: We report: 1) a correlation between the induction of each CYP gene family and the O2 yield; 2) support to an observation reported previously that the tumor promoting ability of CYP inducers is mainly mediated by the O2; and 3) the description of a method for nitroxide mediated O2 detection in vivo. CONCLUSION: These findings could open the way for using electron spin resonance in diagnostic practice.

Perez-Trepichio AD, Jones SC. **Evaluation of a novel nimodipine delivery system in conscious rats**

that allows sustained release for 24 h. J Neurosci Methods 1996;68(2):297-301.
CBAC COPYRIGHT: CHEM ABS Methodologies that allow prolonged drug administration in animal models, while minimizing surgery and anesthesia, are an important contribution towards studies in awake conditions. Com. available drug delivery systems like pellets can be customized for the evaluation of exptl. therapies with minimal or no discomfort to animals. The objective was to evaluate pharmacokinetic and physiol. parameters after s.c. implantation of rapid 24 h release nimodipine pellets in rats for their potential use as a delivery system for stroke therapeutics. A day prior to the study Sprague-Dawley rats were anesthetized (halothane, N2O, O2) for femoral vessel cannulation and later returned to their cages. On the day of the study, the rats were briefly anesthetized (identical regimen as before), and assigned to two groups: nimodipine (NP) and placebo (PL). The NP 15 mg group showed a significant decline of 10% in MABP from base line to 24 h post implantation. All NP animals achieved at least 83% of their highest plasma concn. at 1 h and 94% at 3 h. A high degree of correspondence between the plasma and brain concns. of nimodipine was present. Although a significant drop in MABP was obsd. the drop was no greater than 10% in 24 h. Plasma nimodipine levels for the 15 mg animals were within the cerebrovascular effective range. This is the first report to show that 24 h release nimodipine pellets s.c. implanted in rats are a reliable delivery system that allows rapid rise and const. nimodipine plasma levels. Therefore, 24-h release pellets are a suitable alternative to other delivery systems like osmotic pumps.

Rogers MJ, Chilton KM, Coxon FP, Lawry J, Smith MO, Suri S, Russell RG. **Bisphosphonates induce apoptosis in mouse macrophage-like cells in vitro by a nitric oxide-independent mechanism**. J Bone Miner Res 1996;11(10):1482-91.
Bisphosphonates (BPs) are an important class of antiresorptive drugs used in the treatment of bone diseases, including osteoporosis. Although their mechanism of action has not been identified at the molecular level, there is substantial evidence that BPs can have a direct effect on osteoclasts by mechanisms that may lead to osteoclast cell death by apoptosis. BPs can also inhibit proliferation and cause cell death in macrophages in vitro. We have now shown that the toxic effect of BPs on macrophages is also due to the induction of apoptotic, rather than necrotic, cell death. Morphological and biochemical features that are definitive of apoptosis (chromatin condensation, nuclear fragmentation, and endonuclease-mediated internucleosomal cleavage of DNA) could be identified in mouse macrophage-like J774 and RAW264 cells, following treatment with 100 microM pamidronate, alendronate, and ibandronate for 24 h or more. Clodronate was much less potent, even at 2000 microM, while 2000 microM etidronate did not cause apoptosis. Apoptosis was not due to increased synthesis of nitric oxide and could not be prevented by inhibitors of nitric oxide synthases. Since macrophages, like osteoclasts, are particularly susceptible to BPs, these observations support the recent suggestion that the mechanism by which BPs inhibit bone resorption may involve osteoclast apoptosis. Furthermore, the macrophage-like cell lines used in this study may be a convenient model with which to identify the molecular mechanisms by which BPs promote apoptosis in osteoclasts. Induction of macrophage apoptosis by BPs in vivo may also account, at least in part, for the anti-inflammatory properties of BPs as well as the ability of BPs to cause an acute phase response.

Schnatter AR, Bird MG, Cox LA, Herrick RF. **Defining optimal exposure assessment methods and metrics for epidemiological studies of exposure of petroleum distribution workers to benzene**.

Occup Hyg 1996:3(1-3):155-60.
An adaptation of a published, physiologically based pharmacokinetic model (PBPK) for benzene (71432), was applied to a study of concentration, duration and exposure intermittency effects, to illustrate application of PBPK modeling in valid exposure metric determination for epidemiological studies. Exposure scenarios typically encountered by petroleum distribution workers were studied. The model was based on data from human subjects, voluntarily exposed to a range of benzene concentrations and monitored through blood, urine and exhaled air for a range of measured outputs. Model outputs were fit to empirical data from humans mice and rats. Benzene and total metabolite concentrations in blood and bone marrow, were mapped over an administered concentration, time series. The model predicted total benzene metabolite concentration in bone marrow, over typical exposure ranges. The model also predicted that the contributions of exposure concentration and duration would be approximately equal over the typical concentration ranges encountered by workers. Short term (within a day) exposure discontinuities played no role in prediction of total bone marrow metabolite concentrations. The authors conclude that the model's predictions are within pharmacodynamic limitations, but they might be effective for determination of exposure metrics for epidemiological studies.

Shieh HL, Hansen H, Zhu J, Riedel H. **Activation of conventional mammalian protein kinase C isoforms expressed in budding yeast modulates the cell doubling time--a potential in vivo screen for protein kinase C activators**. Cancer Detect Prev 1996;20(6):576-89.
Conventional mammalian protein kinase C (PKC) isoforms alpha, beta 1, and gamma were expressed in Saccharomyces cerevisiae and resulted in a differential increase in the yeast doubling time in response to distinct classes of PKC activators. Mutants were created in the regulatory domain of PKC alpha to map the interaction with the different activators. The macrocyclic lactone bryostatin 5 preferentially regulated PKC alpha activity through the second cysteine-rich sequence (CYS2) of Cl, while regulation by the diterpene ester mezerein displayed strong preference for the first cysteine-rich sequence (CYS1) of Cl. The phorbol esters phorbol-12-myristate-13-acetate (PMA) and 12-deoxyphorbol 13-phenylacetate 20-acetate (dPPA) regulated PKC enzymatic activity equally potently via CYS1 or CYS2 albeit at reduced levels compared with native PKC alpha. For the diterpene ester ingenol-3, 20-dibenzoate and the indol alkaloids (-)-7-octyl-indolactam V and (-)-indolactam V, no responses were observed for mutants lacking either CYS1 or CYS2 whereas native PKC alpha activity was regulated. These in vivo results were complemented by in vitro binding and catalytic assays which showed correlation between PKC enzymatic activity and the cell growth characteristics. The observed phenotype can be exploited to screen natural compounds in vivo for their PKC regulatory potential and to map the underlying interactions.

Xie YS, Bodnaryk RP, Fields PG. **A rapid and simple flour-disk bioassay for testing substances active against stored-product insects**. Can Entomol 1996;128(5):865-75.
BIOSIS COPYRIGHT: BIOL ABS. A rapid and simple flour-disk bioassay was developed to assay biologically active substances on several species of stored-products insects. The speed, simplicity, and parsimony of the bioassay derive from a single-step mixing of the test substance in aqueous solution with flour. Aliquots (100 muL) of the stirred suspension are then pipetted onto a polystyrene Petri dish using an Eppendorf pipettor and allowed to dry at room temperature overnight to produce uniform flour

disks containing the test substance. After equilibration at 30 : 1 C and 70 : 5% relative humidity for 24 h, the disks are individually weighed and transferred to Petri dishes with weighed stored-products insects. After 3 days, the remainder of the disk and the living insects are weighed again for calculations of food consumption, utilization, growth, and mortality. Based on the flour-disk bioassay, the neem-based insecticide, Margosan-O, significantly reduced consumption, growth, feeding, and dietary utilization in Cryptolestes ferrugineus (Stephens), Sitophilus oryzae (L.), and Tribolium castaneum (Herbst) in a dose-dependent manner. Margosan-O also caused mortality, but the species differed widely in sensitivity, C. ferrugineus being the most sensitive and T. castaneum the least. The mortality of C. ferrugineus and S. oryzae was a consequence of both toxic and antifeedant (starvation) effects, but mortality of T. castaneum was caused entirely by its toxic action. Using a whole-kernel bioassay, it was found that Margosan-O caused a dramatic reduction in the F1 progeny of all three species.