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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

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

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

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Suggestions and comments are welcome.

CANCER/TUMOR TOXICITY

1

Wright SC, Zhong J, Larrick JW. INHIBITION OF APOPTOSIS AS A MECHANISM OF TUMOR PROMOTION. FASEB Journal 1994;8(9):654-660.

Recent evidence supports the concept that tumor growth in vivo depends on evasion of normal homeostatic control mechanisms that operate through induction of cell death by apoptosis. This study tested the hypothesis that a common property shared by known or suspected tumor promoters is the ability to block the process of apoptosis. A total of 10 tumor promoters were tested and all were found to inhibit DNA fragmentation and cell death of 7 different cell lines triggered into apoptosis by diverse agents. Resistance to apoptosis could be induced rapidly (within 1 h) by treating with relatively high concentrations of promoters. However, low physiological concentrations of promoters could also induce complete resistance to apoptosis after prolonged exposure (5-15 days of culture). Like tumor promotion in vivo, promoter-induced resistance to apoptosis was reversible after culturing in the absence of promoter. These findings provide new insight into the mechanism of tumor promotion and suggest a novel in vitro screening assay to detect new tumor-promoting agents in the environment.

2

Pinzani P, Gerli A, Carotti S, Sestini R, Gelmini S, Orlando C, Mini E, Mazzei T, Pazzagli M. TUMOR CHEMOSENSITIVITY TEST MONITORED BY BIOLUMINESCENCE. G Ital Chim Clin 1993; 18(3):211-17.

In vitro tumor chemosensitivity assays (TCA) have been developed which are based on measurement of radioactive precursor incorporation, differential staining, fluorescent cytoprint, and capability of forming colonies. The measurement of ATP by bioluminescence can represent another biochem. based parameter of drug-induced cytotoxicity. We evaluated a TCA test based on intracellular ATP quantitation by the firefly luciferin-uciferase system both in cell lines and tumor primary cell cultures. The test is performed on polypropylene microplates using few thousands of cells per well (1.0.times.103 for cell lines, 3.0.times.104 for tumor primary cell cultures) in proprietary medium to inhibit nonmalignant cell survival. Each microplate is used to test 4-6 drugs or drug combinations at 6-7 concns. in duplicates or triplicates. After 5-7 days of cell incubation, ATP is then measured on 50 muL of cell ext. using the Berthold LB9500 luminometer. The method is rapid, sensitive, and practicable, so that measurements can be performed using a very low no. of cells without interferences due to biol. matrix and extn. soln. A comparison with a clonogenic assay gave comparable results when applied to the study of 4 human T-lymphoblast cell

lines (CCRF-CEM) with different sensitivity to methotrexate. The TCA has been used to evaluate chemotherapeutic drug sensitivity of cultured tumor cell line ME180 (cervical squamous carcinoma cells, ATCC HTB 33), as well as on clin. specimens of an endometrial carcinoma. The TCA test seems to have application for both drug development and selection of therapy for primary tumors, drug resistant tumors, and tumors of unknown primary site.

3
Cahan MA, Walter KA, Colvin OM, Brem H. CYTOTOXICITY OF TAXOL IN VITRO AGAINST HUMAN AND RAT MALIGNANT BRAIN TUMORS. Cancer Chemother Pharmacol 1994;33(5):441-4.

Taxol is a novel antitumor alkaloid that has shown clin. activity against several tumors, including ovarian and breast carcinoma and melanoma. To evaluate taxol's potential as a therapy for malignant brain tumors, we measured the sensitivity of four human (U87, U373, H80, and D324) and two rat (9L, F98) brain-tumor cell lines to taxol. The cells were exposed to taxol in vitro using a clonogenic assay. Log cell kill (LD90) occurred at concns. of 42 (9L), 25 (F98), 19 (H80), 7.2 (U373), 9.1 (U87), and 3.9 nM (D324) when cells were continuously exposed to taxol for 6-8 days. The human cell lines were uniformly more sensitive to taxol than were the rat lines. The duration of exposure had a significant effect on taxol's cytotoxicity. When cells were exposed to taxol for 1 h the LD90 increased to 890 nM for the 9L rat line and 280 nM for the human U373 line. On the basis of these results, we conclude that taxol has significant potency in vitro against malignant brain tumors and that the activity occurs at concns. of taxol that have previously been shown to be effective for several tumors against which the drug is currently being evaluated clin.

4
Kikuchi H, Ujiie S, Itoh Y, Wakui A. ORGANOID FORMING METHOD. A NEW IN VITRO CHEMOSENSITIVITY TEST. Gan to Kagaku Ryoho 1994; 21(3):351-62.

In 1988, Friedman and coworkers reported a new chemosensitivity test using "organoids"(epithelial cell aggregates) which had a high plating efficiency and short assay period. The authors recognized this test to be useful and studied it exptl. Human tumor xenografts maintained in the subcutis of BALB/c nu/nu mice were minced into cell aggregates and filtered, then resuspended in enriched NCTC 135 cell culture medium which contained no serum. Petri dishes were coated with a mixt. of collagen-I and bovine serum albumin and dried for an hour. The cell aggregates were seeded in these coated dishes and cultured in a condition of

low O2 tension (3% O2). Plating efficiency at 24 h in cultures of three tumors were 20.8.+-.2.6% on SC-6-JCK (stomach), 37.8.+-.3.8% on NS-8 (stomach) and 27.2.+-.1.5% on PAN-1-RITC (pancreas), resp. The cell no. of each organoid increased until 72 h in culture, although the organoid no. of each dish decreased slightly. Flow cytometrical measurement of total DNA content in dishes showed that the amt. of human DNA increased more rapidly than that of mouse DNA which was derived from interstitial and infiltrative cells. This culture system appeared to allow a selective growth of epithelial cells. Subsequently, some drug sensitivity was tested using this system. SC-6-JCK tumor is sensitive to mitomycin C (MMC), although resistant to adriamycin (ADM) in a test using nude mice (s.c.-i.p. system). Organoids were formed from this tumor and chemosensitivity was tested against MMC and ADM from the viewpoint of change in the organoid no. in each dish. After one hour of drug exposure, only a part of the cells in organoids was affected. On the contrary, after an exposure of 24 h, the ADM-treated group showed the same results as the MMC-treated group. Hence this test was considered to become more appropriate by counting not the organoid no. but the total cell no. in the dish.

5
Van Ark-Otte J, Peters GJ, Pizao PE, Keepers YP, Giaccone G. IN VITRO SCHEDULE-DEPENDENCY OF EO9 AND MILTEFOSINE IN COMPARISON TO STANDARD DRUGS IN COLON CANCER CELLS. Int J Oncol 1994; 4(3):709-15.

In vitro screening of new antitumor drugs is often limited to fast chemosensitivity assays. The potency of drugs is studied by measuring growth inhibition at the end of the assay after a fixed drug exposure time. In this study, the human colon cancer cell lines HT29 and SW620, were exposed to drugs for 1 h, 24 h (followed by culture in drug-free medium), 72 h, and for 72 h with drug renewal every 24 h. Growth inhibition was evaluated at 48 and 72 h after initial drug addn., using the sulforhodamine B (SRB) assay. Chemosensitivity profiles of the investigational drugs EO9, a bioreductive alkylator, and the ether lipid miltefosine (HPC), were compared to those of drugs with different mechanisms of action: doxorubicin, cisplatin (DDP) and 5-fluorouracil. HPC displayed recovery from growth inhibition, in both cell lines, after drug exposures of 1 and 24 h. DDP showed an increase for both cell lines ($p < 0.05$) of growth inhibition when drug was being refreshed every 24 h, compared to 72 h continuous drug exposure. Doxorubicin, 5-fluorouracil and EO9 were more potent in SW620 cells. These data suggest that more initial information can be obtained from drug screening assays, when both continuous and short-term drug exposures are studied, instead of one fixed drug exposure time, and indicates that daily renewal of drugs may reveal possible drug stability

and availability problems.

CARCINOGENESIS
6
Leboeuf RA, Kerckaert GA, Isofort RJ, Aardema MJ, Gibson DP, Cody DB, Brauninger R. MULTI-STAGE TRANSFORMATION OF SHE CELLS A BIOLOGICALLY RELEVANT IN VITRO MODEL FOR IDENTIFYING CHEMICAL CARCINOGENS. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):129.

No abstract.

7
Eastmond DA. CHROMOSOMAL MECHANISMS IN ENVIRONMENTAL CARCINOGENESIS. U. S. Department of Agriculture/Cooperative State Res Ser FEDRIP Database, National Technical Information Service (NTIS), Springfield, VA; 1994.

Study the involvement of chromosomal mechanisms in carcinogenesis induced by environmental and agricultural chemicals. APPROACH: Initially, efforts will focus on the metabolism and genotoxicity of two important model agents, benzene and o-phenylphenol which are likely to exert their carcinogenic effects through chromosomal mechanisms. These investigations will rely on antibody and molecular cytogenetic techniques to rapidly assess structural and numerical aberrations in cell culture and animal systems. The feasibility of using DNA probes to detect chromosomal aberrations in human populations will also be determined by comparing aberration frequencies in the peripheral blood lymphocytes and exfoliated urothelial cells of smokers and nonsmokers. -- <PR> PROGRESS: Significant highlights of our research on chromosomal mechanisms of carcinogenesis over the past year are as follows. 1) Using multicolor fluorescence in situ hybridization techniques, we have developed a new method to detect hyperdiploidy and chromosome breakage in interphase human cells. The usefulness of this approach has been demonstrated in vitro using cell culture techniques and in vivo in a pesticide-exposedhuman population. We have also developed a similar approach which allows us to determine whether micronuclei originating in the bone marrow of mice following chemical exposure results from chromosome loss or breakage. 2) Both chromosome loss and breakage have been shown to be common types of genotoxic events occurring in cell cultures following the exposure of cells to hydroquinone, t-butylhydroquinone, 4,4'-methylene bis(2-chloroaniline) and a superoxide anion radical generating system. These chromosomal alterations may play a role in the carcinogenic effects induced by various quinone-forming and arylamine carcinogens.

CARDIOTOXICITY

8

Legrand J-J, Pingot V. HIERARCHICAL IN VITRO TESTING STRATEGY FOR EVALUATION OF CARDIAC TOXICITY. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994; 7(2):146.

No abstract.

CELL CULTURE

9

Hanthamrongwit M, Reid WH, Courtney JM, Grant MH. 5-CARBOXOFLUORESCEIN DIACETATE AS A PROBE FOR MEASURING THE GROWTH OF KERATINOCYTES. Human Experimental Toxicol 1994;13(6):423-427.

There is a requirement for a convenient and reliable method for evaluating the growth rate of human keratinocytes cultured on collagen-based substrates. Therefore, three methods of determining cell growth were first used to quantify the growth rate of the well-characterized L929 mouse fibroblast cell line on tissue culture plastic and the results compared. The methods used were the measurement of total cell protein, cell counting using an electronic Coulter counter and a fluorimetric assay employing 5carboxyfluorescein diacetate (CFDA). The CFDA assay showed the highest correlation with seeding density of the L929 cells, and the lowest standard deviations. If was the most rapid and convenient method for processing large numbers of samples. Only viable cells can deacetylate the non-fluorescent CFDA to carboxyfluorescein, which is fluorescent and accumulates inside the cells. Therefore, the assay specifically quantifies only viable cells. Subsequently, this assay has been successfully applied to the measurement of human keratinocyte growth rate on collagen gels and sponges. We have demonstrated that keratinocytes grow equally well on gels and sponges, and that media containing low calcium concentrations (0.09 mm) favor rapid proliferation of keratinocytes. Our results show that the CFDA assay is an accurate, reliable and convenient method for quantifying cell growth in vitro. It is particularly valuable when growing cells on optically opaque substrata, such as collagen sponges, where growth cannot be monitored daily by microscopy.

10

Wolfgang GH I, Vernetti LA, MacDonald JR. ISOLATION AND USE OF PRIMARY ADRENOCORTICAL CELLS FROM GUINEA PIGS, DOGS AND MONKEYS FOR IN VITRO TOXICITY STUDIES. Toxicol Methods 1994;4(3):149-60.

A method was developed to obtain enriched populations of zona fasciculata cells from the adrenal glands of guinea pigs, dogs, and monkeys. Adrenocortical cells (ADC) in primary culture were shown to maintain viability and cellular morphol., with an estd. .gtoreq. 80% of cultures being zona fasciculata cells. Three known adrenal toxicants, PD 132301-2, o,p'-DDD, and aminoglutethimide (AG) were tested in this in vitro system. Neutral red (NR) uptake was used as a marker of cell viability and cortisol prodn. was measured to assess ADC function. NR uptake following 24 h of treatment with PD 132301-2 (10 muM) was 32, 31, and 53% of control in guinea pig, dog, and monkey cultures, resp. Similarly, o,p'-DDD (100 muM) decreased NR uptake to 32, 40, and 69% of control. AG (300 muM) decreased NR uptake by 50% only in dog ADC. Cortisol prodn. was evident in cells from all three species with rates being highest in monkey, followed by the dog and guinea pig. Cortisol prodn. was decreased following treatment with all three toxicants. Decreases paralleled loss of viability in PD 132301-2-treated cultures from all three species and in o,p'-DDD-treated cultures from guinea pig and dog. In contrast, decreased cortisol prodn. preceded any change in viability in o,p'-DDD-treated cultures from monkey, and in AG-treated cultures from all species. Cytotoxic responses to adrenal toxicants of varied structure and mechanisms of action suggests that the ADC cultures from these three species may be useful in toxicol. screening or for investigating mechanisms of adrenocortical toxicity.

11
van 't Klooster GA, Woutersen-van Nijnanten FM, Blaauboer BJ, Noordhoek J, van Miert AS. APPLICABILITY OF CULTURED HEPATOCYTES DERIVED FROM GOAT, SHEEP, AND CATTLE IN COMPARATIVE DRUG METABOLISM STUDIES. Xenobiotica 1994;24(5):417-28.

1. Using trimethoprim (TMP), scoparone (SCOP), ethylmorphine (EtM), 1-naphthol (1-N) and phenol red (PhR) as test substrates, biotransformation activities were investigated in cultured hepatocytes from male and female rat, male and female goat, and female sheep and cattle. 2. As compared with rat hepatocytes, the total culture cytochrome P450 content was relatively well maintained in ruminant hepatocytes. In 72 h, it decreased to approximately half the initial content, whereas in rat hepatocytes only 30% was maintained. In ruminant hepatocytes, sulphation of 1-N remained fairly stable, glucuronidation of PhR decreased gradually, and glucuronidation of 1-N increased during the 72-h culture period. 3. Oxidative metabolism of TMP was rapid in goat and sheep hepatocytes, as compared with rat hepatocytes, reflecting species differences in TMP pharmacokinetics in vivo. In contrast with rat hepatocytes, 6-O-demethylation was by far the major pathway of scoparone

metabolism in ruminant hepatocytes. The glucuronidation and sulphation activities were similar among the species. 4. In goat liver cells, sex differences in some oxidative biotransformations were observed, females being more active than males. In rat hepatocytes, a reverse sex difference was observed. 5. In conclusion, cultured hepatocytes from agricultural target species appear a useful in vitro model to study comparative metabolism of veterinary drugs and other xenobiotics. Comparing rat and ruminant, sex and species differences and similarities in drug metabolism can be observed that reflect the in vivo situation.

12
Genestie I, Morin JP, Vannier B, Leclaire J, Lorenzon G. PRIMARY CULTURES OF RABBIT PROXIMAL TUBULE CELLS ON COLLAGEN IV-COATED POROUS MEMBRANES. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):123.

No abstract.

13
Holzmann H, Kippenberger S, Ramirez-Bosca A, Bereiter-Hahn J, Bernd A. CELL AND TISSUE CULTURE MODELS IN DERMATOLOGY: THE ESTABLISHED MODELS AT FRANKFURT DERMATOLOGY CENTRE. Hautarzt 1994;45(5):304-312.

In the course of the Third Frankfurt Talks, for the first time a congress in dermatology was dedicated exclusively to cell and tissue culture models. The complexity of a whole organ, in this case the skin, could be reduced to single aspects without losing the holistic context. Ways of managing this were discussed on an interdisciplinary level by dermatologists, physiologists, pharmacologists and biologists. The results are also expected to be useful to the clinician. Focus points of in vitro investigations for dermatology are wound closure models and the use of in vitro skin for transplantation in the therapy of nonhealing ulcers and vitiligo. As an alternative to animal experiments, cultures of human cells are gaining increasing influence in drug testing. The effect of glucocorticosteroids on normal skin fibroblasts, keratinocytes and permanent cell lines is discussed as an example, Additionally, basic events such as differentiation and ageing have been modelled in cell cultures of melanocytes and keratinocytes. Mechanical stress, UV radiation and nicotine are discussed as inductors.

14
Czich A, Bartsch I, Dogra S, Hornhardt S, Glatt HR. STABLE

HETEROLOGOUS EXPRESSION OF HYDROXYSTEROID SULPHOTRANSFERASE IN CHINESE HAMSTER V79 CELLS AND THEIR USE FOR TOXICOLOGICAL INVESTIGATIONS. Chemico-Biological Interactions 1994; 92(1-3):119-128.

Various benzylic alcohols are metabolically activated to electrophilic, potentially mutagenic and carcinogenic sulphuric acid esters. The involved sulphotransferases are not expressed in the cell lines in culture which are commonly used for mutagenicity testing. The liver of adult female rats is very efficient in the bioactivation of 1-hydroxymethylpyrene. The major enzyme involved was purified and identified as hydroxysteroid sulphotransferase a. Its cDNA was stably expressed in Chinese hamster V79 cells, which are particularly suited for the quantitative detection of various types of mutations and other genotoxic and cytotoxic effects. The mRNA, protein and enzyme activity levels in the constructed cell lines (V79rSTa-I and V79rSTa-2) were measured, and the cells were also used in mutagenicity and cytotoxicity investigations with benzylic alcohols. 1-Hydroxymethylpyrene,

9-hydroxymethylanthracene and 6-hydroxymethylbenzo(a)pyrene showed enhanced cytotoxicity in V79rSTa-1 and V79rSTa-2 cells, as compared with sulphotransferase-deficient control cells. In addition, 1-hydroxymethylpyrene induced sister chromatid exchanges, and 6-hydroxymethylbenzo(a)pyrene induced gene mutations in V79rSTa-1 cells. We intend carrying out more investigations with other chemicals on these cell lines. Their advantages, as compared with systems with external metabolising systems, include the formation of the active metabolites within the target cell, as in ST-proficient cells in vivo, eliminating the problems which may result from restricted intercellular transport of reactive and ionized sulphuric acid conjugates. Furthermore, cells expressing other sulphotransferases, including human enzymes, may be constructed and used for comparative investigations.

15
Arterburn LM, Overton RM, Stickler EE, Zurlo J. CHARACTERIZATION OF A RAT HEPATOCYTE CULTURE SYSTEM FOR TOXICITY TESTING. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):136.

No abstract.

16
Wallace KA, Harbell JW, Mun G, Janus J, Curren RD. THE USE OF NORMAL HUMAN KERATINOCYTES AND THE NEUTRAL RED UPTAKE BIOASSAY TO

ASSESS MEIC COMPOUNDS NUMBER 31-50. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994; 7(2):158.

No abstract.

17
Pizzoferrato A, Ciapetti G, Stea S, Cenni E, Arciola CR, Granchi D, Savarino L. CELL CULTURE METHODS FOR TESTING BIOCOMPATIBILITY. Clin Mater 1994;15(3):173-90 (62 REFS)

Cell culture systems may be of value in testing the biocompatibility of prosthetic materials before they are introduced into clin. use. In recent years, in vitro methods for assaying biomaterials have gained in importance owing to the growing concern over the use of animals for biomaterials testing. A no. of methods for measuring both the cytotoxicity and the specific cytocompatibility of different materials are available. The literature on the use of cell culture models in evaluating biocompatibility and reports on the personal experience of the authors, who have been using cell culture systems for many years and for different purposes are discussed.

18
Helferich W. IMPROVE FOOD SAFETY THROUGH DISCOVERY AND CONTROL OF NATURAL AND INDUCED TOXICANTS AND ANTITOXICANTS. U. S. Department of Agriculture/Cooperative State Res Ser FEDRIP Database, National Technical Information Service (NTIS), Springfield, VA; 1994.

Develop biological and chemical assays for detecting and identifying natural and induced toxicants in raw and processed foods. Identify and investigate mechanisms of action of food-born anti-toxicants that may reduce risks to human health. APPROACH: Develop biosensor based assays using cellular receptor responsive enhancer DNA sequences in conjunction with a reporter plasmid to generate stably transfected mammalian cell lines. These cells will be used to determine the relative content of these biologically active chemicals in foods and feeds. In subsequent studies, natural toxicants and anti-toxicants will be evaluated using established rodent models and cell culture studies. Experiments will involve biochemical and molecular toxicology measurements such as: changes in organ weights, enzyme induction analysis and expression of genes involved in the observed responses. -- <PR> PROGRESS: The long range objectives are to identify foodborne chemicals which are biologically active. We utilized the estrogen responsive MCF-7 cells in a variety of assays designed to evaluate estrogenic

activity. Our first experiments characterized ER activity in the MCF-7 cells.The KD of estradiol to this receptor is 250pM based and Scatchard analysis. This cell line will be suitable for conducting the estrogenic functional assays. In order to conduct these functional assays estrogen response elements were cloned into a recombinant plasmid containing the MMTV promoter (lacking the glucocorticoid responsive enhancers) to drive expression of the luciferinase gene. We have transfected MCF-7 cells witha similar plasmid containing the CAT reporter gene under control of EREs & shown an increase in expression of the CAT activity of 533% & 333% for estradiol & diethylstilbestrol, respectively. We have also shown that the soy phytoestrogen, genistein, will bind to the ER (KD = 100X that of estradiol). When genistein is fed to ovariectomized rats a 5 fold increase in uterine weight was observed. Addditionally, mRNA for an estrogen responsive gene was also increased in animals fed the phytoestogen.Thus, the in vitro & in vivo data correlate well. Our plans for next year are to prepare stable transformants expressing estrogen-dependent LUC activity in MCF-7 cells. These recombinant lines should provide excellent model systems for detection & estimation of the relative estrogenicpotency of a given food sample.

19
Bhunia AK, Steele PJ, Westbrook DG, Bly LA, Maloney TP, Johnson MG. A SIX-HOUR IN VITRO VIRULENCE ASSAY FOR LISTERIA MONOCYTOGENES USING MYELOMA AND HYBRIDOMA CELLS FROM MURINE AND HUMAN SOURCES. Microb Pathog 1994;16(2):99-110.

An in vitro cell culture assay using myeloma cells and hybrid lymphocytes was developed which detected pathogenic Listeria strains in just 6 h. Three separate hybridoma cell lines, murine Ped-2E9 and EM-7G1 and human RI.37 and murine myeloma NS1 cells, proved equally sensitive in responding to virulent Listeria species. Listeria monocytogenes along with other Listeria spp., collected from food and clinical sources, were inoculated at 10(8) cfu/ml into a suspension of Ped-2E9 (10(6)/ml). Pathogenic Listeria spp. killed 80% of hybridoma cells by 4 h, as determined by trypan blue exclusion test. Conversely, none of all nonpathogenic Listeria spp. killed the hybridoma cells. Ped-2E9 cells exposed to three strains of L. monocytogenes strains showed 96-97.5% death in 6 h measured by trypan blue staining and release of 91-97% of lactate dehydrogenase (LDH) enzyme. RI.37 cells showed similar results. A multiplicity of exposure (MOE) of 100 L. monocytogenes to 1 hybridoma cell or of 10:1 killed about 80% of the hybridoma cells in 4 or 6 h respectively. The in vitro virulence assay of L. monocytogenes with hybridoma cells compared favorably with the immunocompromised mouse model, yielding results in 6 h instead of 3 days. Intracellular L. monocytogenes and L. innocua were not recovered from Ped-2E9 hybridoma cells after 2 or 4 h of exposure. However, attachment of both L.

monocytogenes and L. innocua cells on Ped-2E9 cell surfaces were observed under epifluorescence microscopy. Direct contact of hemolysin positive L. monocytogenes with hybridoma cells is essential to cause death, since hybridoma cells were not killed when they were separated from the growing bacteria by a 0.45 microns filter.

20
Budunova IV, Willaims GM. CELL CULTURE ASSAYS FOR CHEMICALS WITH TUMOR-PROMOTING OR TUMOR-INHIBITING ACTIVITY BASED ON THE MODULATION OF INTERCELLULAR COMMUNICATION. Cell Biol Toxicol 1994;10(2):71-116.

The ability of chemicals with tumor-promoting or tumor-inhibiting activity to modulate gap junctional intercellular communication is reviewed. The two most extensively used types of assays for screening tests are (1) metabolic cooperation assays involving exchange between cells of precursors of nucleic acid synthesis and (2) dye-transfer assays that measure exchange of fluorescent dye from loaded cells to adjacent cells. About 300 substances of different biological activities have been studied using various assays. For tumor promoters/epigenetic carcinogens, metabolic cooperation assays have a sensitivity of 62% and dye-transfer assays 60%. Thirty percent of DNA-reactive carcinogens also possess the ability to uncouple cells. The complete estimation of the predictive power of these assays could not be made because the majority of the substances studied for intercellular communication effects in vitro have not yet been studied for promoting activity in vivo. Both metabolic cooperation assays and dye transfer assays respond well to the following classes of substances: phorbol esters, organochlorine pesticides, polybrominated biphenyls, promoters for urinary bladder, some biological toxins, peroxisome proliferators, and some complex mixtures. Results of in vitro assays for such tumor promoters/nongenotoxic carcinogens, such as some bile acids. some peroxides, alkanes, some hormones, mineral dusts, ascorbic acid, okadaic acid, and benz(e)pyrene, do not correlate with the data of in vivo two-stage or complete carcinogenesis. Enhancement of intercellular communication was found for 18 chemicals. Among these, cAMP, retinoids, and carotenoids have demonstrated inhibition of carcinogenesis. We examine a number of factors that are important for routine screening, including the, requirement for biotransformation for some agents to exert effects on gap junctions. We also discuss the mechanisms of tumor promoter and tumor inhibitor effects on gap junctional Permeability, including influences of protein kinase activation, changes in proton and Ca2+ intracellular concentrations, and effects of oxy radical production.

21
Morshed KM, Jain SK, McMartin KE. ACUTE TOXICITY OF PROPYLENE GLYCOL: AN ASSESSMENT USING CULTURED PROXIMAL TUBULE CELLS OF HUMAN ORIGIN. Fundamental Applied Toxicol 1994;23(1):38-43.

The present study assesses the acute toxicity of propylene glycol (propane-1,2-diol, PD) using cultured human proximal tubule (HPT) cells as the model. Confluent monolayers of HPT cells were treated with 0-263 mm (0-2%) isotonic solutions of racemic, sinister, and rectus PD (rac-, S-, and R-PD, synonym: DL-, L-, and D-PD, respectively). Release of lactate dehydrogenase (LDH) and of preloaded 51Cr as markers of in vitro toxicity, were increased by PD in a time- and concentration-related manner,

suggesting significant PD-induced damage of the HPT cell membrane. These toxic effects reached an apparent maximum within 2 hr. Further studies were performed to determine whether adverse effects of PD may occur prior to development of membrane damage. Because significant membrane damage was not apparent during the first 15 min of PD exposure, cellular release of lactate and accumulation of glucose were studied after preincubating cells in PD (treated) or pH 7.4 buffer (control) for 10 min. PD significantly increased the subsequent release of lactate by concentration- and enantiomer-dependent mechanisms with R-PD being the most potent agent. PD also inhibited the Na+-independent, carrier-mediated glucose accumulation without significant effects on the Na+-dependent pathway. These data suggest a rapid onset of cellular toxicity even when the plasma membrane integrity and viability remained apparently normal. The present studies show significant toxic effects of PD and suggest that a primary culture of HPT cells may be useful in evaluating the toxicity of xenobiotics.

22
MacDonald C, Vass M, Willett B, Scott A, Grant H. EXPRESSION OF LIVER FUNCTIONS IN IMMORTALISED RAT HEPATOCYTE CELL LINES. Human Experimental Toxicol 1994;13(6):439-444.

The differentiated hepatic function of two rat liver cell lines, P9 and SV40RH1, immortalized by transfection with SV40 DNA has been investigated in terms of the glutathione synthesis, and the activities of gamma-glutamyltransferase, glutathione-S-transferase and UDP-glucuronosyltransferase. SV40RH1 is a highly differentiated cell line at early passage, but the expression of some aspects of its differentiated phenotype is unstable and some functions are lost by passage 12-13. P9 is a less-well differentiated cell line, with relatively stable expression of functions between passages 4 and 13. In terms of differentiated function both cell lines represent c marked improvement over primary cultures of rat hepatocytes which de-differentiate

rapidly within 24-48 h in culture. This retention of liver function in proliferating cell lines offers the opportunity to use such cells in in vitro toxicological studies.

CYTOTOXICITY

23

Korting HC, Herzinger T, Hartinger A, Kerscher M, Angerpointner T, Maibach HI. DISCRIMINATION OF THE IRRITANCY POTENTIAL OF SURFACTANTS IN VITRO BY TWO CYTOTOXICITY ASSAYS USING NORMAL HUMAN KERATINOCYTES, HaCaT CELLS AND 3T3 MOUSE FIBROBLASTS: CORRELATION WITH IN VIVO DATA FROM A SOAP CHAMBER ASSAY. J Dermatol Sci 1994;7(2):119-29.

Cell cultures have been proposed as a promising model for local tolerance testing. This study evaluated the cytotoxic effects of surfactants on early passage normal human keratinocytes, transformed human keratinocytes (HaCaT cells) and Swiss 3T3 embryonic mouse fibroblasts. Cell membrane integrity, as assessed by the release of the vital dye neutral red, and cell proliferation, as assessed by measurement of the total protein content, were both affected in a dose-dependent manner in response to surfactant exposure. There was a close correlation between the dose-response characteristics for the three cell types. Two surfactants exhibited differential effects on membrane integrity and proliferation, and thus no significant correlation was found between the two endpoints. The irritation potential of the surfactants to human forearm skin in vivo was assessed in a soap chamber test using transepidermal water loss and skin redness as quant. endpoints. A comparison between the responses in vivo and in vitro yielded the highest correlation for the neutral red release test on normal keratinocytes. (Abstract truncated at 400 words)

24

Munoz MJ, Castano A, Blazquez T, Vega M, Carbonell G, Ortiz JA, Carballo M, Tarazona JV. TOXICITY IDENTIFICATION EVALUATIONS FOR THE INVESTIGATION OF FISH KILLS: A CASE STUDY. Chemosphere 1994; 29(1):55-61.

A large fish-kill was observed in the river Tajo during the Spring-Summer of 1991. The mortality was first detected between Aranjuez and Toledo, affecting several fish species. Then it was slowly going downstream, affecting only carp (Cyprinus carpio), reaching the Spanish-Portuguese border several months later. Short-term toxicity tests on Daphnia magna and in vitro cytotoxicity tests on RTG-2 cells were used as toxicity monitoring systems in water samples and different water fractions. The fish kill was associated to the outbreak of infectious diseases, spring viremia of carp and saprolegniosis,

related to an increase in the fish's susceptibility due to the presence of a toxic chemical. Bioassay-directed sample fractionations allowed to detect a toxic chemical. HPLC-MS identified the compound as dehydroabietic acid, a resin acid previously described immunotoxic.

25
Hurbankova M, Tilkes F. USE OF THE TEST FOR ESTIMATION OF LDH ACTIVITY AS ONE PARAMETER FOR DEMONSTRATING CYTOTOXICITY OF FIBROUS DUST. Prac Lek 1993;45(4):149-53.

The authors used the in vitro detn. of lactate dehydrogenase (LDH) activity of guinea pig alveolar macrophages after 20-h exposure to fibrous dusts (crocidolite, wollastonite from China, wollastonite from the USA, Supelco, Dolanit) at doses of 100, 200 and 300 mug .times. 10-6 of alveolar macrophages. The results were compared with unexposed control and with non-fibrous dusts (corundum-relatively inert, and DQ12-a highly toxic dust). The decreasing order of toxicity was displayed by DQ12, asbestos-crocidolite, wollastonite-China, wollastonite-USA, corundum, Dolanit and Supelco. LDH values followed a statistically significant concn. gradient in all types of the examd. samples with the exception of Supelco and Dolanit, where the effect were not related to the dose of fibers.

26
Lewis RW, McCall JC, Botham PA, Trebilcock R. A COMPARISON OF TWO CYTOTOXICITY TESTS FOR PREDICTING THE OCULAR IRRITANCY OF SURFACTANTS. Toxicol in Vitro 1994;8(4):867-9.

In vivo rabbit eye tests have attracted criticism on both scientific and ethical grounds. Consequently, there is a need to develop new approaches that still provide the necessary information on eye irritation hazard but that minimize or even avoid the use of whole lab. animals. Cytotoxicity models have been used to predict the ocular irritancy of surfactants, since this class of chems. has an essentially common action on cell membranes which involves membrane disruption. The aim of the present studies was to compare the predictive ability of two in vitro cytotoxicity tests, the K562 and the red blood cell lysis tests, in the assessment of the in vivo eye irritancy of surfactants. The results of these studies on 14 selected surfactant materials showed that the K562 assay was only modestly predictive of the in vivo response, with a specificity of 86% but a sensitivity of only 57%. In contrast, the red blood cell lysis test was more predictive, correctly identifying all irritants tested. In addn., all non-irritant surfactants examd. were predicted and a high (89%) ability rank irritant effect was demonstrated. The red blood cell lysis test could be a

powerful addn. to a testing strategy or pre-screen for the evaluation of surfactant chems.

27
Clothier RH, Morgan SJ, Atkinson KA, Garle MJ, Balls M. DEVELOPMENT OF A FIXED-DOSE APPROACH FOR THE FLUORESCEIN LEAKAGE TEST. Toxicol in Vitro 1994;8(4):883-4.

To answer the question, how toxic is this material, rather than the question more usually asked in in vitro toxicol., at what concn. does this material produce a given degree of toxicity, a fixed-dose approach has been developed with the FRAME fluorescin leakage (FL) test. Recovery from the initial cytotoxic effect was also noted sometimes, after the cells had been maintained in fresh medium for a further 72 h. Chems. rated R41 or R36 in vivo gave a level of FL above 20% immediately, and 66% or more 72 h later (i.e. there was further deterioration, rather than recovery). Acetaldehyde, when duplicated and tested either in Hanks' balanced salt soln. or oil, showed greater toxicity in the oil because of its high volatility. The chems. classified as no-label gave FL values below 12% immediately after exposure, except Brij-35, which showed no further deterioration during the 72-h period in fresh medium.

28
Dickson FM, Lawrence JN, Benford DJ. CYTOTOXICITY OF 12 CHEMICALS OF KNOWN HUMAN AND ANIMAL SKIN IRRITATION POTENTIAL IN HUMAN KERATINOCYTE CULTURES. Toxicol In Vitro 1994;8(4):661-3.

Twelve chems. were selected for cytotoxicity testing, based on a report of a collaborative study (EC Project 86E3-063/86-460) that compared in vitro cytotoxicity in animal cell models with animal irritation and non-invasive human in vivo data. Neutral red (NR) and acid phosphatase (AP) activity were chosen as endpoints of cytotoxicity. AP activity has been shown to be a specific indicator of cytotoxicity in keratinocytes. NR50 values (concn. that produces a 50% redn. in NR uptake compared with controls) and AP(peak) values (concn. at which a peak activity occurs) were calcd. for each chem. Both in vitro assays exhibited good correlations with the animal irritation data. Poor correlations were obtained for comparison of the cutaneous blood flow vol. data. The data presented provide useful information on the relationship between in vivo skin irritation and in vitro exptl. models.

29
Chang JD, Hwang WI. CYTOTOXIC ACTIVITY OF OMEGA-3 FATTY ACID AGAINST CANCER CELLS IN VITRO. Koryo Taehakkyo Uikwa Taehak

Nonmunjip 1993;30(1):21-33.

This study was devised to observe the cytotoxic activity of omega-3 (n-3) fatty acids against human colon (HT-29) and rectum (HRT-18) cancer cells and normal monkey kidney cell (VERO 76) in vitro. Alpha-Linolenic acid and Cod liver oil contg. 25% or 70% n-3 fatty acids were used for this expt. Each cell line was incubated in medium contg. several concn. of alpha-linolenic acid and cod liver oil for 72 h, and then the growth rate and dose-response of each cell were detd., and size distribution and morphol. change of HRT-18 estd. The exptl. results obtained are summarized as follows, 1. The growth rates of HT-29, HRT-18 and VERO 76 were gradually inhibited in proportion to the concn. (below 40 mug/mL) of alpha-linolenic acid in culture medium, then the cell nos. of each cell line were significantly reduced compared with initial seeding cell no. at above 50 mug/mL concn. of alpha-linolenic acid. 2. The growth rate of HT-29 was slightly reduced in medium contg. 100 mug/mL of Cod liver oil contained 25% n-3 fatty acid, compared with control group, but the growth rates of HRT-18 and VERO 76 were similar to that of control group. 3. The cell no. of HT-29 cultured in medium contg. 100 mug/mL of Cod liver oil (contained 70% n-3 fatty acid) maintained the level of initial seeding cell no., but that of HRT-18 and VERO 76 were reduced to 59% and 30% compared with control group after incubation for 72 h. 4. The cell size of HRT-18 incubated in culture medium contg. alpha-linolenic acid (40 mug/mL) and Code liver oil (200 mug/mL) was significantly reduced, compared with that of control group, also, the microscopic shape of HRT-189 was shrinked and distracted. In view of the result, it could be suggested that n-3 fatty acids have strong cytotoxic activity against human rectum and colon cancer cell line in vitro test.

30
Castano A, Vega M, Blazquez T, Tarazona JV. BIOLOGICAL ALTERNATIVES TO CHEMICAL IDENTIFICATION FOR THE ECOTOXICOLOGICAL ASSESSMENT OF INDUSTRIAL EFFLUENTS: THE RTG-2 IN VITRO CYTOTOXICITY TEST. Environ Toxicol Chem 1994;13(10):1607-11.

No abstract.

31
Bondy GS, Armstrong CL, Dawson BA, Heroux-Metcalf C, Neville GA, Rogers CG. TOXICITY OF STRUCTURALLY RELATED ANTHRAQUINONES AND ANTHRONES TO MAMMALIAN CELLS IN VITRO. Toxicol in Vitro 1994; 8(3):329-35.

Anthraquinones and structurally related compds. were cytotoxic to mammalian cell lines using cloning efficiency and MTT redn. as

endpoints. In V79 cells, the concn. of chem. causing 50% inhibition ranged from 0.21 to 21.6 mug/mL for cloning efficiency and from 0.86 to 14.6 mug/mL for MTT redn. The anthrones anthralin and chrysarobin were 4.1 and 3.2 times more toxic, resp., in the cloning efficiency assay than in the MTT assay. In contrast, the anthraquinones danthron and emodin were 2.8 and 2.1 times more toxic, resp., in the MTT assay than in the cloning efficiency assay. Among the four mammalian cell lines tested using the MTT assay, the human leukemia cell line (K562) was the most sensitive to the test chems. In contrast, anthraquinone toxicity was reduced in rat hepatoma (H411E) cultures. In general, structures with carbonyl groups in positions 9 and 10 on the anthracene skeleton (anthraquinones) were less toxic than structures with carbonyl groups in position 9 only (anthrones). Toxicity was also influenced by the position of hydroxy substituents on the tricyclic skeleton. The results suggested that in vitro cytotoxicity assays are useful in elucidating the relationships between structure and biol. activity for anthraquinones and related compds.

32
Riva MC, Lopez D. USE OF IN VITRO CYTOTOXICITY TESTS IN EVALUATION OF EFFECTS OF ENVIRONMENTAL POLLUTANTS. Bol Intexter Inst Invest Text Coop Ind 1993;104:59-64. (12 REFS)

No abstract.

33
Saito H, Shigeoka T. COMPARATIVE CYTOTOXICITY OF CHLOROPHENOLS TO CULTURED FISH CELLS. Environ Toxicol Chem 1994; 13(10):1649-50.

In vitro cytotoxicity of chlorophenols to Cypinus carpio brain (CCB) cells derived from carp and Oryzias latipes fin (OLF-136) cells derived from medaka was examd. with the neutral red (3-amino-7-dimethylamino-2-methylphenazine hydrochloride) incorporation assay. Results were compared with previous cytotoxicity data of chlorophenols to goldfish (Carassius auratus) scale (GFS) cells derived from the goldfish. There were excellent correlations between the 24-h NR50 values of chlorophenols toward the three kinds of cells ($r^2 > 0.94$).

34
Barile FA, Dierickx PJ, Kristen U. IN VITRO CYTOTOXICITY TESTING FOR PREDICTION OF ACUTE HUMAN TOXICITY. Cell Biol Toxicol 1993; 10(3):155-62.

Expts. were performed in the absence or presence of increasing

doses of the test chem. during an 18- to 24-h incubation. Comparison of the cytotoxic concns. confirms previous independent findings that the exptl. IC50 values are more accurate predictors for human toxicity than equiv. toxic blood concns. (HETC values) derived from rodent LD50s. In addn., there were no conclusive statistical differences among the methods. It is anticipated that, together, these procedures can be used as a battery of tests to supplement or replace currently used animal protocols for human risk assessment.

35
Mark H FL, Naram R, Pham T, Shah K, Cousens LP, Wiersch C, Airall E, Samy M, Zolnierz K, et al. A PRACTICAL CYTOGENETIC PROTOCOL FOR IN VITRO CYTOTOXICITY AND GENOTOXICITY TESTING. Annals Clinical Laboratory Science 1994;24(5):387-395.

In vitro cytogenetics has been established as a valid method for evaluating the genotoxic potential of chemical agents. Armstrong et al have described a simple, quantitative approach to in vitro cytotoxicity and genotoxicity testing by using Chinese hamster ovary (CHO) cells. This approach can also be sensitive and repeatable in an inter-laboratory setting, a prerequisite for routine testing of compounds suspected of having genotoxic properties. In the present study, cytotoxicity was evaluated by the parameter of mitotic index (MI). Genotoxicity is measured by the chromosome aberration (Abs) assay as described by Armstrong et al using CHO cells. The basic analytic principles proposed were extended to include human lymphocytes. Sister chromatid exchange (SCE) analysis was used to establish an additional endpoint. Mitomycin C (MMC), an established clastogen, was used as the model compound for protocol validation. Dose response curves for MI and Abs in CHO cells were found to be consistent with those reported by Armstrong et al. Results from our extended study on lymphocytes and using SCE analysis were analogous. Our experience is that this standardized approach is indeed sensitive and reliable and can serve as a basis for an inter-laboratory testing program.

36
Ryan JA, Hightower LE. EVALUATION OF HEAVY-METAL ION TOXICITY IN FISH CELLS USING A COMBINED STRESS PROTEIN AND CYTOTOXICITY ASSAY. Environmental Toxicol Chem 1994;13(8):1231-1240.

All organisms, from bacteria and yeast to humans, respond to physical and chemical stressors by increasing the synthesis of a small group of "cellular stress proteins." We have developed a simple in vitro system for quickly screening environmentally relevant stressors to detect stress-induced proteins that are good candidates for biomarkers. Polyacrylamide gel

electrophoresis was used to detect stressor-induced, concentration-dependent changes in cellular stress protein levels in two fish cell culture systems, whereas simultaneous in vitro neutral red uptake cytotoxicity assays measured the stressor's effect on cellular physiology. There was a direct concentration-dependent relationship between sublethal cytotoxic effects and the increases in stress protein levels. Increases of 50 to 200% were detected in stress proteins from desert topminnow, Poeciliopsis lucida, hepatoma-derived cell cultures exposed to cadmium (six proteins) or copper (four proteins). Three proteins showed similar increases in winter flounder, Pleuronectes americanus, kidney cell cultures exposed to the same stressors. Increases in the evolutionarily conserved heat-shock protein hsp70 were detected in each experiment; its level increased with increasing stressor concentrations.

37
Evans EJ. CELL DAMAGE IN VITRO FOLLOWING DIRECT CONTACT WITH FINE PARTICLES OF TITANIUM, TITANIUM ALLOY AND COBALT-CHROME-MOLYBDENUM ALLOY. Biomaterials 1994;15(9):713-717.

Fibroblastic cells in vitro were exposed to powders of titanium, titanium-aluminium-vanadium alloy and cobalt-chrome-molybdenum (Co Cr Mo) alloy, either in direct contact with the cells or separated from the cells by a microporous membrane. Fine particles of all the materials reduced cell growth when in direct contact with cells, but only the finest particles of Co Cr Mo alloy caused cell damage through the microporous membrane. This provides further evidence that there is a mechanism of cell damage in vitro which depends on a direct interaction between cells and particles and is largely independent of the chemical nature of the particle.

38
Wakuri S, Izumi J, Sasaki K, Tanaka N, Ono H. CYTOTOXICITY STUDY OF 48 MEIC CHEMICALS BY COLONY FORMATION AND ATP CONTENT ASSAYS. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):176.

No abstract.

39
Ekwall B, Clemedson C, Barile F, Calleja MC, Castell J, Chesne C, Clothier R, Curren R, Dierickx P, et al. COMPARISON BETWEEN HUMAN SKIN IRRITANCY AND IN VITRO CYTOTOXICITY FROM 77 SYSTEMS FOR THE FIRST 12 MEIC CHEMICALS. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore,

Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):156.

No abstract.

40
Nakamura M, Imai K. CELL RECOVERY TEST FOR EXAMINING CYTOTOXICITY OF BIOMATERIALS. World Congress on Alternatives and Animal Use in the Life Sciences, Batlimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):125.

No abstract.

41
Knebel JW, Aufderheide M, Emura M. COMPARISON OF BIOLOGICAL EFFECTS OF DIFFERENT POLYCYCLIC AROMATIC HYDROCARBONS IN LUNG CELLS OF HAMSTER AND RAT IN VITRO. Toxicol Lett 1994; 72(1-3):65-72.

The cytotoxicity and frequencies of transformation induced by 5 environmental polycyclic arom. hydrocarbons (PAH) in hamster (M3E3/C3) and rat (WRB K3) lung cells were compared. Both cell strains investigated here retain major metabolic characteristics of the target cells in vivo and are thus able to effectively metabolize, i.e. activate, PAH. Cytotoxic effects of the carcinogen were detd. in colony-forming assays and the PAH tested induced dose-dependent cytotoxic responses in the M3E3/C3 and WRB cells. They could then be classified into strong and weak cytotoxicity. Compared to the hamster cell system, the WRB cells were generally shown to be more sensitive. The transforming capacity of the compds. was detd. by a soft agar colony formation assay detecting cells with anchorage independency (AI). All PAH investigated induced transformation to AI growth in both cell systems. The transforming activity of the PAH, relative to benzo[a]pyrene (B[a]P) as a ref. substance, was detd. to facilitate their ranking. This order of transforming potency appears to be similar to that obsd. in animal studies.

42
Villarreal ML, Alvarez L, Alonso D, Navarro V, Garcia P, Delgado G. CYTOTOXIC AND ANTIMICROBIAL SCREENING OF SELECTED TERPENOIDS FROM ASTERACEAE SPECIES. J Ethnopharmacol, 1994;42(1):25-9.

Twelve pure compds. originally obtained through a systematic chemotaxonomical study with Mexican plants of the Asteraceae, were subjected to a cytotoxic and in vitro antimicrobial screening. Three different cell lines in culture (KB, KB-VI and P388) were used in the cytotoxicity assay, while antimicrobial

activity was tested against Gram-pos. and Gram-neg. bacteria, as well as Candida albicans. Of the twelve terpenoids tested, only taraxasterol showed antimicrobial activity against Staphylococcus aureus. The significant cytotoxic activity exhibited by five sesquiterpene lactones, and the moderate cytotoxicity of an eudesmane, is discussed.

43
Flaskos J, McLean WG, Hargreaves AJ. THE TOXICITY OF ORGANOPHOSPHATE COMPOUNDS TOWARDS CULTURED PC12 CELLS. Toxicol Letters 1994;70(1):71-76. (16 REFS)

The cytotoxicity of tricresyl-phosphate (TCP), triphenyl-phosphate (TPP), and paraoxon was studied in-vitro. Rat PC12 pheochromocytoma cells were incubated with up to 1,000 micrograms per milliliter (microg/ml) TCP, TPP, or paraoxon. Cytotoxicity was assessed by determining the extent of cell death with the trypan-blue dye exclusion test and the antiproliferative activity of the compounds by measuring uptake of tritiated thymidine by the compounds. All three compounds inhibited cell proliferation in a dose dependent manner. The doses causing 50% inhibition of proliferative activity were TCP 15microg/ml, TPP 250microg/ml, and paraoxon 100microg/ml. TCP at 10microg/ml and TPP and paraoxon at 100microg/ml caused significant increases in cell lethality. Because it was the most toxic compound, TCP was evaluated further by adding 1microg/ml to PC12 cells in the presence of 150 nanograms per milliliter nerve growth factor. The effect on the ability of the cells to maintain neurite outgrowths was evaluated for up to 5 days. TCP significantly inhibited neurite outgrowth after 2 and 5 days exposure. The authors conclude that the in-vitro toxicity shown by the compounds does not agree with their in-vivo toxicity. The greater in-vitro toxicity of TCP may reflect an effect on cells that is related to mechanisms involved in organophosphate induced delayed neurotoxicity.

44
Csoka K, Larsson R, Tholander B, Gerdin E, de la Torre M, Nygren P. CYTOTOXIC DRUG SENSITIVITY TESTING OF TUMOR CELLS FROM PATIENTS WITH OVARIAN CARCINOMA USING THE FLUOROMETRIC MICROCULTURE CYTOTOXICITY ASSAY (FMCA). Gynecol Oncol 1994; 54(2):163-70.

The automated fluorometric microculture cytotoxicity assay (FMCA) is based on the measurement of fluorescence generated from cellular hydrolysis of fluorescein diacetate (FDA) to fluorescein by viable cells after a 72-hr culture period in microtiter plates. The FMCA was adopted for chemosensitivity testing of tumor cells from patients with ovarian carcinoma. Thirty-seven

samples of solid tumors and malignant effusions were obtained from 35 patients at diagnosis or relapse. Tumor cells from solid samples and effusions were prepared by enzymatic digestion and centrifugation, respectively, followed by Percoll or Ficoll purification. The fluorescence was proportional to the number of cells/well and considerably higher in tumor cells than in contaminating normal cells. The effect of up to 19 cytotoxic drugs was successfully assessed in 70% of the samples and there was a good correlation between drug sensitivity data reported by the FMCA and the DiSC assay performed in parallel. The overall drug sensitivity pattern in vitro corresponded well to the clinical experience. The effect of cisplatin varied considerably between patients and resistance was found also in cases not previously exposed to cytotoxic drugs. The FMCA is a rapid and simple method that seems to report clinically relevant cytotoxic drug sensitivity data in ovarian carcinomas. In the future, this method may contribute to optimizing chemotherapy by assisting in individualized drug selection and new drug development.

45
Abdul-Hussain S, Acosta D. IN VITRO MODEL TO EVALUATE THE CYTOTOXICITY OF TWO GROUPS OF SURFACTANT MIXTURES WITH RAT KERATINOCYTE CULTURES. Toxic Substances Journal 1994;13(1):1-14. (30 REFS)

The potential cytotoxicity induced by surfactant mixtures was evaluated using primary keratinocyte cultures isolated and established from the skin of neonatal rats. Two groups of surfactant mixtures were used; one contained Miranol-HM (14350960) (HMS) and sodium-dodecyl-sulfate (151213) (SDS) in varying concentrations, while the other contained Tween-20 (9005645) in addition to HMS and SDS. Epidermal keratinocytes were isolated from neonatal Sprague-Dawley-rats and cultured. The keratinocyte cultures were exposed to five different concentrations of each surfactant mixture for 1 hour on day three after seeding. Cellular toxicity was assessed at 1 and 24 hours after chemical exposure by determination of lactate-dehydrogenase leakage; additionally, mitochondrial enzymatic activity was assessed at 1 hour via the 3-(4,5-dimethylthiazol-2yl)-2, 5-diphenyl-tetrazolium-bromide reduction assay. Exposure to the two groups of surfactant mixtures resulted in concentration and time dependent responses; the ratio of individual components appeared to be a major determinant of cytotoxicity. Cell injury did not appear to be reversible. The authors conclude that this in-vitro system may be useful in assessing the cytotoxic potential of surfactant mixtures found in consumer products.

46
Yang W, Acosta D. CYTOTOXICITY POTENTIAL OF SURFACTANT MIXTURES EVALUATED BY PRIMARY CULTURES OF RABBIT CORNEAL EPITHELIAL CELLS. Toxicol Letters 1994;70(3):309-318. (28 REFS)

The use of in-vitro toxicity assays employing cultures of rabbit corneal epithelial cells for the assessment of ocular irritancy potential of surfactants was investigated. Primary rabbit corneal epithelial cell cultures were treated with one of two groups of surfactant mixtures for 1 hour and the cells were evaluated for lactate-dehydrogenase (LDH) leakage and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium-bromide (MTT) dye reduction. These results were then compared to ones previously obtained using the same mixtures in the in-vivo Draize eye test. Each of the two surfactant groups resulted in dose dependent increases in LDH leakage and MTT dye reduction with similar rankings seen for the compounds contained in each group using the two different in-vitro assessment methods. In addition, each method gave similar results when delayed cytotoxic effects were examined by measuring LDH leakage and MTT reduction 24 hours after the initial exposure. These results demonstrated between a 20% and 50% increase in LDH leakage for all mixtures tested with the exception of one. Good correlations were between the results of the in-vitro assays and those reported using the in-vivo Draize eye irritation test.

47
Warshawsky D, Reilman R, Cheu J, Radike M, Rice C. INFANUENCE OF PARTICLE DOSE ON THE CYTOTOXICITY OF HAMSTER AND RAT PULMONARY ALVEOLAR MACROPHAGE IN VITRO. J Toxicology and Environmental Health 1994;42(4):407-421.

Silica and ferric oxide are common industrial exposures. Studies have indicated that all commonly occurring forms of crystalline silica can cause fibrotic lung disease. There is evidence to indicate that crystalline silica is carcinogenic in humans who have not developed silicosis, while amorphous silica is not carcinogenic in humans. An important biological response to particles deposited deep in the lung is their engulfment by pulmonary alveolar macrophages (AM). To assess the role of AM in silica-induced lung disease, particle size distribution and surface area of crystalline, gelled, precipitated, and fumed silica, ferric oxide, and aluminum oxide were characterized, the cytotoxicity of the particles to hamster and rat AM in vitro was measured at 0.0-0.5 mg/1 24 and 48 h using dye exclusion procedures. The count medium diameter for aluminum oxide, ferric oxide, and amorphous silica was equal to or less than 0.38 mum, while for crystalline silica the value was 0.83 mum. The surface areas for the amorphous silicas and the aluminum oxide ranged from 253 to 125 m2/g with gelled silica having the highest value,

the values for crystalline silica and ferric oxide were 4.3 and 10.8 m2/g, respectively. Crystalline silica (1.6%) was detected in the fumed silica, while none was detected in precipitated or gelled silica. With gelled silica, based on the dose of the particle, the viability of the hamster AM decreased to 27% at 0.05 mg and to zero at 0.1 mg at 24 h. At doses of 0.05 and 0.1 mg of crystalline, precipitated, or fumed silica, the percent viability decreased significantly to 76-67% and 51-42%, respectively, and to zero at 0.5 mg. Macrophages viable at 24 h decreased further at 48 h compared with the control culture. The ferric oxide and the aluminum oxide showed minimal to no changes in viability. Similar results for the particles were obtained with rat AM. The results indicate that precipitated and fumed amorphous silica tested at equivalent doses are equally as toxic to AM lavaged from two species of rodents as crystalline silica; gelled silica is more toxic than crystalline. Ferric oxide and aluminum oxide are noncytotoxic in this system. The results of this study indicate that the dose as well as the surface area and surface characterization are important determinants in the cytotoxicity of hamster and rat AM to these particles.

48
Lin P, Bernstein IA, Vaughan FL. FAILURE TO OBSERVE A RELATIONSHIP BETWEEN BIS-(BETA-CHLOROETHYL)SULFIDE-INDUCED NAD DEPLETION AND CYTOTOXICITY IN THE RAT KERATINOCYTE CULTURE. J Toxicol Environment Health 1994;42(4):393-405.

It has been proposed that the activation of poly(ADP-ribose) polymerase (Papirmeister et al., 1985), which results from the presence of strand breaks in bis-(beta-chloroethyl)sulfide (BCES) damaged DNA, causes depletion in the level of nicotinamide adenine dinucleotide (NAD) leading to cell death. This hypothesis has now been evaluated in the primary submerged culture of rat keratinocytes. The DNA content, the viable cell number, and the proliferative capability (measured by thymidine incorporation) of the culture were all reduced 48 h after exposure to 10 muM BCES. However, the total NAD level, that is, NAD+ plus NADH, was not changed at a dose of BCES lower than 50 muM. This observation was the same in both proliferating and early differentiating cultures. To further test this hypothesis, the modifying effect of inhibiting poly(ADP-ribose) polymerase on cytotoxicity in BCES-exposed cells was investigated. After exposure to 250 muM BCES, the NAD level was reduced to approximately 26 pmol/mug DNA. This value was increased to 34-49 pmol/mug DNA at both 24 and 48 h postexposure when the cultures were incubated in medium supplemented with 1-10 mM nicotinamide. Nevertheless, the decrease in the DNA content of the culture was not reversed. These results suggest that in the rat keratinocyte culture exposed to BCES, depletion of NAD is not a prerequisite for cell death.

DENTAL TOXICITY
49
Tasaka Y, Horasawa N, Takahashi S, Uematsu T, Hasegawa T. BIOLOGICAL EVALUATION OF DENTAL METALS - TOXIC EFFECT OF METAL CATIONS. Matsumoto Shigaku 1994;20(1):64-9.

The toxic effects of 4 metal cations released generally from dental casting alloys were evaluated by corrosion test and in vitro cytotoxicity. Corrosion tests were conducted on 4 pure metal plates (Cu, Zn, Ag, Pd), and immersed in human resting saliva for 3 wk at 37.degree.. Quant. anal. of the metal cations released into the saliva was performed by inductively coupled plasma-emission spectrometry. In vitro cytotoxicity was examd. using L-929 cell lines. The cell line to be tested was adjusted to 2 .times. 104 cells/mL in growth medium contg. copper and zinc ions at various concns. Cytotoxicity was detd. to have occurred if a 50% inhibitory concn. was found when compared to controls (IC50). The results obtained were as follows. The amt. of copper ion released into human saliva was 274 mug/cm2. This was equal with the amt. of nickel ions released from some Ni-Cr alloys. The amt. of zinc ions released was 14 mug/cm2. The release was restrained by white matter deposited on the surface of the metal plate, 3 days later. Silver and palladium were excellent for the inhibition of corrosion to human saliva. The IC50 of the copper ion was 1.28 ppm (20.2 muM) and of the zinc ion it was 10.6 ppm (159 .mu.M).

50
Wataha JC, Hanks CT, Craig RG. THE EFFECT OF CELL MONOLAYER DENSITY ON THE CYTOTOXICITY OF METAL IONS WHICH ARE RELEASED FROM DENTAL ALLOYS. Dent Mater 1993;9(3):172-6.

The effect of cell density (number of cells per unit area of a monolayer culture) on the in vitro cytotoxicity of metal ions which are known to be released from dental materials was investigated. The effects of cell density (1) may explain previous discrepancies in in vitro tests, (2) may be important in wound healing where cell density changes over time, and (3) may help clarify the mechanisms of cytotoxicity of metal ions. Balb/c 3T3 fibroblasts were plated at cell densities ranging from 10,000-80,000 cells/cm2 and were exposed to 8 concentrations of 10 different metal ions. After 24 h, the succinic dehydrogenase activity and DNA synthesis were measured to quantify the cytotoxic effect. Higher cell densities markedly reduced the sensitivity of these fibroblasts to all metal ions except Al+3 and Zn+2, but the magnitude of the reduction was metal dependent. In addition, the DNA synthesis was inhibited more than the succinic dehydrogenase activity for all metal ions except Zn+2.

The unique effect of cell density on each metal ion supported the hypothesis that the effect was not simply caused by a dilution of the number of metal ions per cell. Given these results, the effect of cell density should be carefully selected in in vitro cytotoxicity tests.

DEVELOPMENTAL TOXICITY
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Friedman M, Stevens KL, Blankemeyer JT. DEVELOPMENTAL TOXICITY OF FOOD INGREDIENTS. U. S. Department of Agriculture/Agricultural Research Service. FEDRIP Database, National technical Information Service (NTIS), Springfield, VA; 1994.

Define mechanisms of action of known and newly identified potato and tomato alkaloids, related secondary plant metabolites, and biosynthetic intermediates, using cell membrane integrity and membrane potential assays. Using these assays and a statistical matrix design, evaluate antagonism and synergism of mixtures of alkaloids to establish combinations with lowest toxicities. APPROACH: A biosensor-type instrument will be used to measure the effects of the test compounds on the following parameters: (a) integrity of frog embryo, frog skin, and erythrocyte cell membranes; (b) sodium, potassium, and calcium ion active transport across membranes; and (c) changes in cellular DNA and intracellular pH. The results will provide (a) information on the mechanism of actions of Solanum alkaloids and related compounds at the cellular level, thus facilitating development of improved potato and tomato cultivars; and (b) rapid, low-cost in vitro assays to assess food safety and water quality. Documents SCA with Oklahoma State University. -- <PR> PROGRESS: Previous studies suggested that the glycoalkaloids disruptedmembrane integrity and affected membrane potential of frog embryos. Frog skin is an established model for trans-epithelial active transport ofsodium from the pond side to the serosal side of the skin. To test the effect of glycoalkaloids on sodium active transport, we exposed frog skin to the potato glycoalkaloids alpha chaconine and alpha solanine as well as their aglycone, solanidine, in a specially designed Ussing glass chamber. We found that interstitial short-circuit (ISC), the measure of trans-epthelial active transport of sodium, decreased up to 30 percent at the solubility limit of alpha chaconine. Alpha solanine had a smallereffect, decreasing short-circuit current by 16 percent at the solubility limit. Solanidine produced an inverted response curve becoming less at higher concentrations. The data suggest that a) frog skin is a usefulexperimenta model to evaluate effects of alkaloids at the cellular level, and b) the mechanism of action of the two glycoalkaloids is to modifythe active transport of sodium.

EMBRYOTOXICITY

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Schiffmann D, Degen GH. EFFECT OF INDANYL ANALOQUES OF DIETHYLSTILBOESTROL ON MORPHOLOGICAL TRANSFORMATION OF SYRIAN HAMSTER EMBRYO FIBROBLASTS AND MICRONUCLEI IN VITRO. Toxicol In Vitro 1994;8(3)L:449-454.

Two analogues of the carcinogenic oestrogen diethylstilboestrol (DES), indenoestrol A (IA) and indenoestrol B (IB), have been studied with respect to their genotoxic and cell transforming properties in mammalian cells. The conformation and estrogenicity of IA and IB are very similar, but they differ with respect to their ease of peroxidation and the resulting reactive intermediates: only IA is readily converted to a p-quinone. Both DES derivatives caused morphological transformation of Syrian hamster embryo (SHE) fibroblasts with similar efficiency at concentrations (1 to 10 muM) that did not affect cloning efficiency. In addition, IA and IB (5 to 50 muM) induced micronuclei in the same cell system. These micronuclei were detectable as early as 1-3 hr after a 5-hr treatment with IA or IB. Such a time course is characteristic of compounds that cause mitotic disturbances. Indomethacin, an inhibitor of prostaglandin H synthase (the enzyme responsible for oxidation of DES in target cells), did not affect the frequency of micronuclei induced by IA or by IB. From these data we conclude that peroxidative metabolism resulting in quinone formation is not a prerequisite for the genotoxicity of these compounds. In addition to DES and several of its analogues the indanyl derivatives now also have been shown to be active in both of these short-term assays, suggesting that the in vitro micronucleus formation and morphological cell transformation are causally related events.

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Hunter E S III, Tugman JA, Sulik KK, Sadler TW. EFFECTS OF SHORT-TERM EXPOSURE TO ETHANOL ON MOUSE EMBRYOS IN VITRO. Toxicol In Vitro 1994;8(3):413-421.

The adverse developmental effects of ethanol consumption have been documented in humans and in animal models. In animal models, the organ system affected by ethanol administration is dependent on the point in gestation at which the xenobiotic is administered. Previous studies have shown that an exposure of 24-48 hr beginning at the early somite stage in rodent conceptuses alters neural tube closure in vitro. However, the concentration and time dependency of this effect have not been fully defined. Whole embryo culture was therefore used to expose 3-6-somite mouse conceptuses (ICR strain) to ethanol at 300, 450, 600 and 800 mg/dl. The higher concentrations were selected to approximate the peak serum ethanol concentrations that have been

shown to be teratogenic in vivo. A 24-hr exposure produced a concentration-dependent increase in neural tube defects (NTDs) and concomitant growth retardation. When shorter exposure periods were used (8, 10, 12 or 20 hr) the incidence of NTDs was dependent on the ethanol concentration and exposure period. At the 600 and 800 mg/dl concentrations an exposure of 8 hr or more produced NTDs, but shorter periods (4 and 6 hr) did not affect neural tube closure when evaluated at the end of a 24-hr culture period. At the 450 mg/dl concentration a 20-hr exposure induced NTDs, but a 12-hr exposure to this level did not. Exposure of conceptuses to ethanol for periods similar to their half-life in vivo did not induce NTDs and the highest concentration produced only a trend towards a reduction in protein content. When the incidence of NTDs was plotted against the area under the time and concentration curve (AUC) the correlation coefficient was 0.5779. An analysis of covariance indicated that the relationships between NTDs and AUC were similar at the 300 and 450 mg/dl concentrations and also at the 600 and 800 mg/dl concentrations. In contrast, the relationships between embryonic protein content and AUC did not differ at the 300, 450 and 600 mg/dl concentrations, but all differed from that at the 800 mg/dl level. These results indicate that ethanol-induced NTDs do not appear to be due solely to embryonic

growth retardation. Additionally, ethanol-induced neural tube defects are a function of duration of exposure as well as of peak serum concentration.

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Heuer J, Graeber IM, Pohl I, Spielmann H. AN IN VITRO EMBRYOTOXICITY ASSAY USING THE DIFFERENTIATION OF EMBRYONIC MOUSE STEM CELLS INTO HEMATOPOIETIC CELLS. Toxicol in Vitro 1994;8(4):585-7.

The earliest identified state of a specific cell in mammalian embryonic development is the embryonic stem cell (ES cell). The murine ES cell line D3 was used to establish conditions that allow a reproducible differentiation of ES cells in culture. The development of hematopoietic cells in semi-solid medium parallels to some extent the onset of hematopoiesis in the developing embryo. In Me cellulose cultures, erythropoiesis is obsd. at day 7 to day 8 of culture. An inhibitory influence of retinoic acid is obsd. both on blood cell development and on myocardial cell development. In contrast, retinoic acid induces the development of nerve and skeletal muscle cell differentiation in D3 ES cells.

Further optimization of the culture conditions for ES cell differentiation will facilitate the use of ES cells for embryotoxicity testing in vitro.

55
Takagi M, Sakonju L, Otoi T, Hamana K, Suzuki T. POSTTHAW VIABLIITY OF THE INNER CELL MASS OF IN VITRO-MATURED/IN VITRO-FERTILIZED BOVINE EMBRYOS FROZEN IN VARIOUS CRYOPROTECTANTS. Cryobiology 1994;31(4):398-405.

A study was conducted to examine the viability of inner cell mass (ICM) cells of frozen-thawed in vitro-matured (IVM)/in vitro-fertilized (IVF)-derived embryos using various cryoprotectants. Expanded blastocysts were frozen and thawed in 1.4 M glycerol with 0.25 M sucrose (GL), 1.6 M propylene glycol (PG), 1.8 M ethylene glycol (EG), or 1.3 M ethylene glycol monomethyl ether (EME) as cryoprotectants using a one-step method. After thawing, the embryos were cocultured for 24 h with cumulus cells in TCM199. Embryos which were viable after thawing and developed beyond the blastocyst stage were treated by immunosurgery and differential fluorochrome staining for ICM cell counts. Overall, there were no significant differences in the development to blastocyst stage after 24 h culture in each cryoprotectant ($P < 0.05$, chi 2 analysis). The viability of ICM cells of frozen-thawed embryos with each cryoprotectant was lower (GL, 72.7%; PG, 67.8%; EG, 77.5%; EME, 74.7%) than that of unfrozen embryos (84.4%). In the case of PG as a cryoprotectant, viability of ICM cells was significantly lower than that of unfrozen embryos ($P < 0.05$, ANOVA analysis). Our results suggest that the viability of ICM cells of frozen-thawed bovine embryos tend to be lower than that of unfrozen embryos irrespective of the cryoprotectants used. PG was significantly more toxic to the ICM cells compared with the other cryoprotectants.

56
Gregotti CF, Kirby Z, Manzo L, Costa LG, Faustman EM. EFFECTS OF STRYENE OXIDE ON DIFFERENTIATION AND VIABILITY OF RODENT EMBRYO CULTURES. Toxicol Appl Pharmacol 1994;128(1):25-35.

Epidemiological investigations on the reproductive and developmental outcomes of rubber and plastic industry workers have focused on the potential developmental toxicity of styrene, an important occupational chemical, and its metabolite, styrene oxide. We have examined the developmental toxicity of styrene oxide using two in vitro culture systems: micromass cell cultures and whole embryo culture (WEC). Further, we have compared the effects of styrene oxide in both culture systems to the developmental toxicity of styrene in the micromass system. The ability of styrene oxide to affect the differentiation of rat embryo midbrain (CNS) and limb bud (LB) cells was compared to general cytotoxicity over 5 days in high-density micromass cultures. The IC50 for differentiation was 9.2 mug/ml (76 muM) for CNS and 6.7 mug/ml (56 muM) for LB. The LC50 for

cytotoxicity was 9.6 mug/ml (80 muM) and 27.5 mug/ml (228 muM) for CNS and LB, respectively. The values for CNS sensitivities suggest that inhibition of differentiation is probably a consequence of high levels of cytotoxicity. In contrast, effects of styrene oxide on LB endpoints of differentiation were evident at concentration levels which had minimal effects on cell viability. Styrene alone or in the presence of an exogenous monooxygenase system had minimal effects when tested at concentrations 4-12 times the highest IC50 values seen with styrene oxide alone. In whole embryo culture experiments, styrene oxide produced growth retardation and embryo malformations (primarily neural) with an MC50 value of 20 mug/ml (167 muM). The LC50 value for styrene oxide was approximately 1.7-fold higher (33.2 mug/ml, 276 muM). Our in vitro studies suggest that further evaluations of the relationship of developmental toxicity and generalized cytotoxicity of styrene and its metabolite are needed, especially given the low concentrations at which effects were seen.

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Takashima H, Ogawa M, Nagao T, Mizutani M. WHOLE EMBRYO CULTURE FROM THE EGG CYLINDER STAGE IN RATS. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994; 7(2):127.

No abstract.

GASTROINTESTINAL TOXICITY
58
Zucco F, De Angelis I, Vincentini O, Rossi L, Steinkuhler C, Stammati A. POTENTIAL USE OF THE HUMAN INTESTINAL CELL LINE CACO-2 IN TOXICOLOGIC INVESTIGATION. In Vitro Toxicol 1994; 7(2):107-112.

Caco-2 is an established cell line derived from a human colon adenocarcinoma. It is able to express in long-term culture some differentiated functions of the small intestine. We have further characterized these cells for specific brush border enzymes, for enzymatic activities related to the (de)toxifying pathways, and for the epithelial barrier formed, when the cells are cultured on filters. Moreover, we have used this model to study the toxic effect of furazolidone (FZ), a food contaminant. The integrity of the cellular barrier has shown to be impaired, due to the cell death. The effect is more relevant when the cells are exposed from the apical side. The sensitivity of these cells and their capability to give specific responses to the adverse effects make them an appropriate model to study the ingested compounds' toxicity.

GENOTOXICITY

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Zolotareva GN, Akinshina LP, Loginova NS, Kapinosova VP. EXAMINING INTERFERON INDUCERS AS GENE MODULATORS IN THE IN VITRO AND IN VIVO SYSTEMS. Khim-Farm Zh 1993;27(1):6-11.

The antimutagenic activity of synthetic and two natural interferon inducers was established by the gene mutation recording method in the Salmonella microsomes by Eims' test. The interferon inducers have protective effects on both microorganisms and mammals, decreasing the mutagenic effects of solafur. The protective effects of interferon inducers against a genetic damage caused by drugs inducing mutations may be further used to construct the optimal schemes for drug therapy when genotoxic drugs are applied in clin. practice.

60

Cooper A, Fentem J, Balls M. INHIBITION OF INTERCELLULAR COMMUNICATION: AN IN VITRO ASSAY FOR DETECTING POTENTIAL TUMOR PROMOTERS. Toxicol in Vitro 1994;8(4):535-7.

The effects of noncytotoxic concns. of 13 chems. on gap junctional intercellular communication (GJIC) in human keratinocytes and WB 344 cells were detd. using a scrape-labeling method. The chems. were selected according to their reported tumor-promoting effects in either the skin or liver, or their known skin irritancy potential. The in vitro assay was able to discriminate between genotoxic and nongenotoxic chems. and, with keratinocyte cultures, ranked correctly a series of phorbol esters with regard to their reported tumor-promoting potencies in vivo. The data obtained support the suggestion that measurement of the inhibition of GJIC in vitro may provide a means to distinguish substances that have different tumor-promoting potencies.

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Jeng JH, Kuo ML, Hahn LJ, Kuo MY P. GENOTOXIC AND NON-GENOTOXIC

EFFECTS OF BETEL QUID INGREDIENTS ON ORAL MUCOSAL FIBROBLASTS IN VITRO. J Dent Res 1994;73(5):1043-9.

To understand the role of betel quid (BQ) in the pathogenesis of oral submucous fibrosis (OSF) and oral cancer, the authors used DNA damage, cytotoxicity, and cell proliferation assays to study the pathobiol. effects of aq. exts. of three BQ constituents [betel nut (Areca catechu, BN), inflorescence of Piper betel (IPB), and lime], one BN alkaloid (arecoline), and one BN

polyphenol [(+)-catechin] on cultured oral mucosal fibroblasts. Exts. of BN and IPB induced DNA strand break formation in a dose-dependent manner. Exts. of BN and IPB, (+)-catechin, and arecoline decreased cell survival and proliferation in a dose-dependent manner. However, aq. ext. of lime (50-800 mug/mL) increased cell proliferation by 20-40%. These results indicate that BQ contains not only genotoxic and cytotoxic agents, but also compds. which stimulate cell proliferation. These compds. may act synergistically in the pathogenesis of OSF and oral cancer in BQ chewers. In addn., five anti-oxidants [glutathione (GSH), cysteine, mannitol, catalase, and superoxide dismutase (SOD)] were tested for their protective effects against the cytotoxicity of BQ constituents. GSH (1.95 and 2.6 mmol/L) and cysteine (4 and 8 mmol/L) prevented the arecoline-induced cytotoxicity. In contrast, mannitol, catalase, and SOD did not decrease the arecoline-induced cytotoxicity. These results indicate that thiol depletion, but not the attack of oxygen free radicals, could be the mechanism for arecoline cytotoxicity. GSH could also protect cells from the cytotoxicity of IPB ext. Increasing dietary intake of GSH-rich foods or dietary supplements of GSH may have chemopreventive potential to reduce BQ-assocd. oral lesions.

62
Nagayama I, Nagayama M, Iida T, Hirakawa H, Matsueda T, Masuda Y. EVALUATION OF GENOTOXIC EFFECTS OF ORGANOCHLORINE COMPOUNDS RETAINED IN HUMAN BODY USING IN VITRO TEST SYSTEMS. Organohalogen Compd 1993;13(Dioxin '93, 13th International Symposium on Chlorinated Dioxins and Related Compounds):149-52.

In order to evaluate the genotoxic effects of organochlorine compds. in vitro, the authors prepd. the mixt. of PCDDs, PCDFs, and co-PCBs, which very resembled their contamination in healthy people in their compn., and investigated the effects of the mixt. on the induction of both micronuclei and sister chromatid exchanges which have frequently been utilized as indicators of genotoxicity. Results were discussed.

63
Dhillon VS, Singh JR, Singh H, Kler RS. IN VITRO AND IN VIVO GENOTOXICITY EVALUATION OF HORMONAL DRUGS v. MESTRANOL. Mutat Res 1994;322(3):173-83.

Genotoxicity of a widely used estrogen, Mestranol, was undertaken using in vitro, in vivo and host-mediated assay with bacteria as indicator organism. Analyses of chromosome aberrations and sister chromatid exchanges (SCEs) in human lymphocytes and chromosome aberrations, micronuclei and sister chromatid exchanges (SCEs) in bone-marrow cells of mice showed the drug to

be capable of attacking the genetic material. However, both Ames Salmonella/S9 assay with and without S9 mix and host-mediated assay using same tester strains of Salmonella, did not show any significant increase/decrease in the His+ revertants.

64
Mersch-Sundermann V, Schneider U, Klopman G, Rosenkranz HS. SOS INDUCTION IN ESCHERICIA COLI AND SALMONELLA MUTAGENICITY: A COMPARISON USING 330 COMPOUNDS. Mutagenesis 1994;9(3):205-24. (168 REFS)

To examine the concordance of two microbial genotoxicity short-term assays, 330 experimental results for the SOS chromotest using tester strain Escherichia coli PQ37 were compared with the results of the Salmonella/mammalian microsome mutagenicity assay with Salmonella typhimurium TA97, TA98, TA100, TA102, TA104, TA1535, TA1537 and/or TA1538. With respect to qualitative features, the concordance between SOS chromotest and Salmonella mutagenicity test results was 86.4% (sensitivity, 78.6%; specificity, 100%; chi 2 = 188.6). None of the non-mutagens (N = 120) were able to induce the SOS system. Additionally, 45 of the 210 S.typhimurium mutagens (21.5%) did not induce the SOS repair system. On closer examination, the majority of these 45 compounds (84%) were mutagens with activities between 0.001 and 10 rev/nmol. Even though the experimental protocols of both systems were not standardized, the correlation coefficient for the experimental results of the two test systems was 0.7 for the 330 chemicals. Except for aliphatic epoxides (r = 0.47), the mutagenicity/SOS induction correlations for congeneric data sets (polycyclic aromatic hydrocarbons, nitroarenes, nitroarenofurans, mycotins) were even better (r = 0.72-0.95). Additionally, computer automated structure evaluation (CASE) analyses of the nature of the structural determinants associated with each endpoint indicate extensive homologies. The data can be taken to indicate that the two phenomena reflect common mechanisms of action.

65
Aquirrezabalaga I, Santamaria I, Comendador MA. THE w/w+ SMART IS A USEFUL TOOL FOR THE EVALUATION OF PESTICIDES. Mutagenesis 1994;9(4):341-346.

Genotype-dependent variability in the response of several Drosophila strains to hexamethylphosphoramide (HMPA) has been studied using the white/white+ (w/w+) somatic mutation and recombination test (SMART). Among the tester strains, there were three wild-type laboratory strains (Leiden-S, Oregon-K and 91-C) and three insecticide-resistant strains (Haag-79, Hikone-R and 91-R). The response to HMPA of larvae from a cross between two

wild-type strains (Leiden-S and Berlin-K) was also measured. The strains have been evaluated in terms of spontaneous frequencies of mosaic eyes, lowest effective dose and dose-response relationship. Strong variability was found among the strains, the best performance to HMPA being obtained with the strain Oregon-K. In addition, a series of pesticides structurally related to HMPA, such as dimefox, hexamethylmelamine, hexazinone, alachlor, CAM, pirimicarb, dimetilan, thiram and methabenzthiazuron have been tested with the Oregon-K strain. Some of these pesticides had already been shown to be genotoxic in other systems, whereas others have either not been tested or gave negative results in in vitro systems. Although genotoxicity was expressed only within a narrow dose range, all pesticides were genotoxic in the w/w+ system with the Oregon-K strain. Thus, these compounds may be a genotoxic hazard to man. These results suggest the suitability of the strain Oregon-K for genotoxicity testing with the w/w+ eye mosaic system, although more information about the performance of this strain with other compounds must be obtained. It is concluded that the w/w+ SMART is an excellent in vivo system able to reveal genotoxicity of promutagens that are difficult to detect with in vitro genotoxicity test systems.

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Glatt H. COMPARISON OF COMMON GENE MUTATION TESTS IN MAMMALIAN CELLS IN CULTURE: A POSITION PAPER OF THE GUM COMMISSION FOR THE DEVELOPMENT OF GUIDELINES FOR GENOTOXICITY TESTING. MutatRes 1994;313(1):7-20.

In gene mutation tests a decision concerning mutations is made on the basis of hereditary functional changes. In terms of the large amount of data available, the most suitable tests for routine testing in mammalian cells in culture are the tests for acquisition of 6-thioguanine resistance in Chinese hamster cells (V79 and CHO) and for acquisition of alpha,alpha,alpha-trifluorothymidine resistance in the mouse lymphoma line L5178Y TK+/- 3.7.2C. The molecular bases, peculiarities, advantages and disadvantages of these systems will be presented. Which system is to be preferred in any particular case depends among other things on the purpose of the study and the extent to which a technically, competent performance of these comparatively exacting tests can be guaranteed.

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Seemayer NH, Hornberg C, Hadnagy W. COMPARATIVE GENOTOXICITY TESTING OF AIRBORNE PARTICULATES USING RODENT TRACHEAL EPITHELIAL CELLS AND HUMAN LYMPHOCYTES IN VITRO. Toxicol Lett 1994; 72(1-3):95-103.

In the authors' study samples of airborne particulates were collected in the heavily industrialized Rhine-Ruhr region utilizing a high vol. sampler HVS 150 (Streohlein Instruments) equipped with glass fiber filters. Chem. substances were extd. from filters with dichloromethane and quant. transferred to DMSO for tissue culture expts. For detection of genotoxicity of ext. of airborne particulates the authors utilized as a sensitive bioassay the induction of sister chromatid exchanges (SCE) in cultures of human lymphocytes and of tracheal epithelial cells of the Syrian golden hamster. The ext. of airborne particulates was added in various concns. to cell cultures of human lymphocytes and hamster tracheal epithelial cells in presence of bromodeoxyuridine for 72 or 48 h, the last 3 h in presence of demecolcine or nocodazole, resp. Ext. of airborne particulates led in both test systems - human lymphocytes and tracheal epithelial cells of the hamster - to a dose-dependent, highly significant induction of sister chromatid exchanges. Very low quantities of substances corresponding to airborne particulates from less than 1 m3 air were highly effective in both cell systems. In comparison, tracheal epithelial cells of the Syrian golden hamster revealed a higher sensitivity showing a steeper increase of sister chromatid exchanges than human lymphocytes.

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Pool-Zobel BL, Lotzmann N, Knoll M, Kuchenmeister F, Lambertz R, Leucht U, Schroeder H-G, Schmezer P. DETECTION OF GENOTOXIC EFFECTS IN HUMAN GASTRIC AND NASAL MUCOSA CELLS ISOLATED FROM BIOPSY SAMPLES. Environmental Molecular Mutagenesis 1994; 24(1):23-45.

To assess genotoxic burdens from chemicals, it is necessary to relate observations in experimental animals to humans. The success of this extrapolation would be increased by including data on chemical activities in human tissues. Therefore, we have developed techniques to assess DNA damage in human gastric and nasal mucosa (GM, NM) cells. Biopsy samples were obtained during gastroscopy from macroscopically healthy tissue of the stomach or from healthy nasal epithelia during surgery. The specimens were incubated for 30-45 min at 37~C with a digestive solution. We obtained 1.5-8and 5-1095%. The cells were incubated in vitro for 1 hr at 37~C with the test compounds added in their appropriate solvents. In GM cells, we studied N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), sodium dichromate (Na2Cr2O7), nickel sulphate (NiSO4), cadmium sulphate (CdSO4), and lindane. In NM cells, lindane was investigated. Each compound was assessed for DNA damaging activity in cells of at least three different human donor samples using the microgel single cell assay. Similar studies were performed with GM and NM cells obtained from Sprague-Dawley rats. We have found human GM cells to be more

sensitive to the genotoxic activity of MNNG than rat GM cells (low effective concentration (LEC) = 0.16 and 0.625 mug/ml for human and rat, respectively). Human cells were also more sensitive to the cytotoxic/genotoxic activity of NiSO4 (LEC = 5 and 19 mumoles/ml for human and rat, respectively). CdSO4 was genotoxic in human GM cells (LEC = 0.03-0.125 mumoles/ml), whereas no dose-related genotoxicity was observed in rat GM at concentrations up to 0.5 mumoles/ml. In contrast, approximately equal responses regarding genotoxicity and cytotoxicity were observed in rat and human GM for Na2Cr2O7 (0.25-1 mumoles/ml). Lindane, however, was genotoxic in three out of four rat GM but not in human GM cells (0.5-1 mumoles/ml), whereas it was active in both rat and human NM cells. Together with other recently published in vivo findings, our results with lindane can be interpreted according to a parallelogram approach. In view of possible human exposure situations and the sensitivities of the two target tissues from both species, the data imply that lindane will pose a health risk to humans by inhalation but not by ingestion.

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Martelli A, Canonero R, Cavanna M, Ceradelli M, Marinari UM. CYTOTOXIC AND GENOTOXIC EFFECTS OF FIVE n-ALKANALS IN PRIMARY CULTURES OF RAT AND HUMAN HEPATOCYTES. Mutat Res 1994; 323(3):121-126. (20 REFS)

The cytotoxic and genotoxic effects of five n-alkanals (propanal (71238), butanal (71363), pentanal (110623), hexanal (111273), and nonanal (124196)) were investigated using primary cultures of human and rat hepatocytes. The hepatocytes were isolated from livers of Sprague-Dawley-rats or from fragments of healthy human liver and plated. Cells were exposed to half log spaced concentrations of the n-alkanals. N-nitrosodimethylamine was used to verify the metabolic competence of the hepatocytes. At the end of 20 hours of exposure, cells were tested. Cytotoxicity was evaluated using the trypan-blue exclusion test and genotoxicity was evaluated by measuring unscheduled DNA synthesis (UDS). Results showed that cytotoxicity increased with carbon chain length and that the cells of the two species responded similarly. However, while the rat hepatocytes showed a mild but significant dose dependent increase of net nuclear grain counts, the human hepatocytes did not. Nonanal at a 3 to 30 millimolar concentration showed the greatest cytotoxicity, but no UDS was induced in either of the cell types. The authors conclude that at the concentrations these n-alkanals reached in the human liver after the lipid peroxidation of ingested food, no genotoxic effects resulted.

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Kopponen P, Torronen R, Maki-Paakkanen J, von Wright A, Karenlampi S. COMPARISON OF CYP1A1 INDUCTION AND GENOTOXICITY IN VITRO AS INDICATORS OF POTENTIALLY HARMFUL EFFECTS OF ENVIRONMENTAL SAMPLES. Arch Toxicol 1994;68(3):167-173. (52 REFS)

The cytochrome-P-4501A1 (CYP1A1) inducing and genotoxic potentials of flyash and natural peat extracts were studied in-vitro. Hexane extracts resulting from combustion of peat (peat flyash) and biosludge (biosludge flyash) and unburned peat were incubated with Hepa1 and H4IIE cells at concentrations ranging up to 100mg/ml. The extent of induction of aryl-hydrocarbon-hydroxylase (AHH) and ethoxyresorufin-O-deethylase (EROD) activity, marker enzymes for the CYP1A1 system, was determined. Genotoxicity was assessed by measuring the ability of the extracts to induce DNA repair in Escherichia-coli-WP2 and sister chromatid exchanges (SCEs) in Chinese-hamster-ovary (CHO) cells. Peat flyash did not induce AHH or EROD activity in either cell type. Biosludge flyash induced AHH and EROD activity in a dose dependent manner, the doses causing 50% induction (ED50s) of these enzymes being 4.9 and 5.8mg/ml in Hepa1 cells and greater than 26mg/ml and 20mg/ml in H4IIE cells, respectively. Unburned peat also induced AHH and EROD activity in both cell types. The ED50s were 0.4mg/ml for both enzymes in Hepa1 cells and 2.0 to 2.7mg/ml for AHH activity and 1.3 to 1.7mg/ml for EROD activity in H4IIE cells. None of the extracts exhibited genotoxicity in the bacterial DNA repair assay. Only biosludge flyash at 100mg/ml caused a significant increase in the SCE frequency in CHO cells. The authors conclude that the CYP1A1 inducing potency of the flyash and peat samples is not correlated with their genotoxicity and CYP1A1 induction might be a useful adjunct to conventional genotoxicity tests that may not detect potentially toxic compounds or environmental mixtures.

71
Fenech M. THE CYTOKINESIS-BLOCK MICRONUCLEUS TECHNIQUE AND ITS APPLICATION TO GENOTOXICITY STUDIES IN HUMAN POPULATIONS. Environmental Health Perspectives 1993;101(Suppl. 3):101-107. (51 REFS)

Studies have confirmed that the cytokinesis block (CB) technique has made the human lymphocyte micronucleus assay (MN) a sensitive indicator of in-vitro radiation exposure in patients who are undergoing fractionated partial body radiotherapy and rodents who were exposed to uniform whole body irradiation. Extensive studies were also performed to determine the spontaneous level of MN in normal human populations and its relationship to various lifestyle factors. A new variation to the CBMN assay was discovered which enabled the conversion of excision repairable

lesions to MN within one cell cycle using cytosine-arabinoside. The slope of the in-vitro dose response curves was increased by a factor of 1.8 for X-rays, 10.3 for ultraviolet (254 nanometers) radiation, and approximately 40 fold for methylnitrosourea with this method. The authors suggest that the CBMN assay can be used not only to measure whole chromosome loss or chromosome breaks, but may also be used to measure excision repair events. The authors suggest that the versatility and simplicity of the CBMN assay together with new developments in automation should make it useful in monitoring exposed populations as well as identifying mutagen sensitive individuals within a population.

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Hartmann A, Speit G. COMPARATIVE INVESTIGATIONS OF THE GENOTOXIC EFFECTS OF METALS IN THE SINGLE CELL GEL (SCG) ASSAY AND THE SISTER CHROMATID EXCHANGE (SCE) TEST. Environmental Molecular Mutagenesis 1994;23(4):299-305.

Sodium arsenite (NaAsO2) and cadmium sulphate (CdSO4) were tested for their ability to induce genotoxic effects in the single cell gel (SCG) assay and the sister chromatid exchange (SCE) test in human blood cultures in vitro. Both metals induced DNA damage in white blood cells that was expressed and detected as DNA migration in the SCG assay. Dose dependent effects were seen for cadmium in concentrations from 5 10-4-1.5ells, a function of dose, revealed that the majority of exposed cells expressed more DNA damage than cells from control cultures and that with increasing length of DNA migration the variability in migration among cells increased as well. Treatment of cells for 2 hr or 24 hr beginning 48 hr after the start of the blood cultures did not increase the SCE frequency in the case of cadmium but caused a small but significant SCE induction with arsenic at the highest concentration. The metal concentrations which could be investigated in the SCE test were much lower due to a strong toxic effect. Metal concentrations which were toxic in the SCE test were without visible effect in the SCG assay. Thus the two endpoints for the determination of genotoxic effects in vitro differed markedly with respect to the detection of genotoxicity induced by metals. These differences and the biological significance of the findings are discussed.

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Bononatti S, Bolognesi C, Degan P, Abbondandolo A. GENOTOXIC EFFECT OF THE CARBAMATE INSECTICIDE METHOMYL: I. IN VITRO STUDIES WITH PURE COMPOUND AND THE TECHNICAL FORMULATION "LANNATE 25". Environmental Molecular Mutagenesis 1994;23(4):306-311.

The carbamate insecticide methomyl and the methomyl-containing technical formulation "Lannate 25" Were tested on whole blood

human lymphocyte cultures. Both products induced dose-dependent increases in chromosome aberrations and micronuclei. Lannate 25 induced DNA damage as measured by the alkaline elution assay and hydroxylation of guanine at the C8 position. Sister chromatid exchanges were not increased significantly with either product. Overall, the technical formulation was more active than the pure compound, when compared at similar concentrations of active principle. Moreover, a different ratio of CREST-positive/CREST-negative micronuclei was observed with the two products, pure methomyl being relatively more active than Lannate 25 in the induction of CREST-positive micronuclei. On the basis of these results, previous evaluations of methomyl as a nongenotoxic compound should be reconsidered.

74
Rosenkranz HS, Klopman G. STRUCTURAL IMPLICATIONS OF THE ICPEMC METHOD FOR QUANTIFYING GENOTOXICITY DATA. Mutat Res 1994; 305(1):99-116. (32 REFS)

The International Commission for Protection against Environmental Mutagens and Carcinogens (ICPEMC) data base of quantitative genotoxicity scores was analyzed by the CASE structure/activity relational expert system and MULTICASE, a derivative of CASE identifying the most important fragments based on a hierarchical approach. The ICPEMC data base contained 113 chemicals, of which 101 were included in this analysis. Three sets of data were generated: agent scores (the overall scores assigned to each chemical based on all of the test results), in-vitro family (all in-vitro test results such as primary DNA damage, gene mutations and chromosomal effects in prokaryotes and cultured eukaryotic cells); and in-vivo family (the contribution of the in-vivo results such as DNA damage, somatic mutations, chromosomal effects, and heritable changes in mammals and Drosophila). The current study demonstrated that the application of the CASE/MULTICASE approach to the total database as well as to the in-vitro and in-vivo subsets of the data base yielded structural determinants which explained over 95% of the chemicals in the data base and accounted also for the weighing factors.

75
Collins AR, Duthie SJ, Dobson VL. DIRECT ENZYMIC DETECTION OF ENDOGENOUS OXIDATIVE BASE DAMAGE IN HUMAN LYMPHOCYTE DNA. Carcinogenesis 1993;14(9):1733-1735. (10 REFS)

A method for the direct measurement of in-vivo oxidative DNA damage in peripheral lymphocytes was described. The technique involved converting oxidized bases to strand breaks using endonuclease-III and detecting the breaks by single cell gel

electrophoresis. The technique was developed using HeLa cells in culture and free radical strand breaks were demonstrated after 5

minutes of incubation with 100 micromolar hydrogen-peroxide. Little damage was apparent 1 hour after cessation of the exposure. Incubation of the peroxide exposed cells with endonuclease-III after lysis demonstrated a marked peroxide dependent increase in DNA damage compared with that seen after incubation in buffer alone; however, no increase in DNA breakage was seen in cells incubated with endonuclease-III but not exposed to peroxide. When this technique was used on lymphocytes isolated from blood samples from normal human donors, many endonuclease-III sensitive sites were identified. DNA break frequencies in human lymphocytes following incubation with endonuclease-III were determined to be 670 breaks/10(12) daltons of DNA. The authors conclude that the technique described may be useful in screening for DNA damage in epidemiological studies, for monitoring radiation effects in cancer therapy, in genotoxicity testing, and for the study of excision repair pathways.

76
Tucker JD, Auletta A, Cimino MC, Dearfield KL, Jacobson-Kram D, Tice RR, Carrano AV. SISTER-CHROMATID EXCHANGE: SECOND REPORT OF THE GENE-TOX PROGRAM. Mutat Res 1993;297(2):101-180. (533 REFS)

The ability of chemicals to induce sister chromatid exchanges (SCE) was reviewed in this second report of the Gene-Tox program. The genetic basis of SCE was described, forming the background by which SCE could be used as indicators of genotoxicity. Test systems were described and included in-vitro SCE analysis, suspension cultures, monolayer cultures, human lymphocyte cultures, in-vivo SCE analysis, in-vivo/in-vitro SCE analysis, and human biomonitoring. Other aspects discussed included an interpretation of data from the literature, selection of papers for inclusion in the review, statistical evaluation methods, controls, criteria for evaluation, and discrimination of positive and negative responses. Statistical approaches to the analysis of SCEs were addressed, as was the presentation of results. The authors conclude that the strength of the various assay systems is based on their sensitivity to detect chemical exposure, the relative ease with which the tests can be conducted in a wide variety of cell lines and tissues, and the inherent ability to produce readily quantifiable data. However, a major problem pertaining to SCEs is the lack of understanding of their biological significance.

HEPATOTOXICITY
77

Sibanda B, Naughton BA, San Roman J, Weintraub JP, Kamali V. CO-CULTURES OF LIVER-DERIVED STROMAL CELLS AND HEPATIC PARENCHYMA ON NYLON SCREEN TEMPLATES EXHIBIT LONG-TERM EXPRESSION OF LIVER SPECIFIC FUNCTIONS AND CAN BE USED TO ASSESS TOXICITY. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):134.

No abstract.

IMMUNOTOXICITY

78

Edwards SM, Donnelly TA, Rheins LA. IMMUNOTOXICITY ASSESSMENT USING AN IN VITRO HUMAN SKIN MODEL. 22nc Annual Meeting of the American Society for Photobiology, Scottsdale, Arizona, USA, June 25-29, 1994. Photochemistry Photobiol 1994;59(Spec. Issue):11S.

No abstract.

79

Campana D. APPLICATIONS OF CYTOMETRY TO STUDY ACUTE LEUKEMIA: IN VITRO DETERMINATION OF DRUG SENSITIVITY AND DETECTION OF MINIMAL RESIDUAL DISEASE. Cytometry 1994;18(2):68-74. (37 REFS)

Modern clin. applications of cytometry include the detn. of the most powerful antileukemic drugs in each patient at the time of diagnosis and the monitoring of residual disease during and off treatment. The precision of in vitro assays to test the susceptibility of cancer cells to cytotoxic drugs depends on the ability to maintain the cells' viability in culture. We found that bone marrow-derived allogeneic stromal cells are crit. to prevent death by apoptosis of acute lymphoblastic leukemia (ALL) cells. Thus, we devised an in vitro drug sensitivity assay in which ALL cells are seeded onto stromal cells and viable leukemic cells are counted at the end of cultures by flow cytometry. Our

preliminary results indicate that this assay is suitable for evaluating the drug sensitivity of leukemic lymphoblasts and testing the antileukemic activity of potentially effective compds. which have not yet been administered to patients with ALL. The identification of immunophenotypes expressed on leukemic cells but absent or extremely rare among normal hematopoietic progenitors allows close monitoring of the effects of drug treatment in vivo. Phenotypes that afford a detection level of 1 leukemic cell among 10,000 normal bone marrow cells have been identified in 90% of cases of T-ALL, 25% of B-lineage ALL, and 40% of acute myeloid leukemia (AML). In several studies, residual disease emerging during continuation therapy or off treatment almost invariably anticipated overt relapse by 1-7

mo. These data indicate the reliability of immunol. techniques to detect occult leukemia. The combined application of drug sensitivity testing and residual disease monitoring may improve the accuracy of clin. management practices, and, ultimately, lead to improved cure rates.

80
Meredith C, Scott MP. ALTERED GENE EXPRESSION IN IMMUNOTOXICOLOGY SCREENING IN VITRO: COMPARISON WITH EX VIVO ANALYSIS. Toxicol in Vitro 1994;8(4):751-3.

The authors have proposed in vitro immunotoxicol. screening systems utilizing cDNA probes to monitor alterations in cytokine expression in defined cell populations. In this study the authors compared responses obsd. following in vitro exposure with responses in cells obtained ex vivo. Peritoneal macrophages and splenic lymphocytes were cultured from C3H mice or PVG rats and exposed to graded concns. of the immunomodulators, biostim, cyclosporin A, azathioprine or tributylin oxide (TBTO). Biostim (1 mug/mL) induced the expression of cytokine mRNAs in peritoneal macrophage cultures and induced a similar spectrum of cytokine expression in macrophages following administration to mice (100 mug/kg i.p.). Cyclosporin A (1 mug/mL) totally inhibited the expression of IL-2 mRNA in murine mixed lymphocyte cultures and induced a 40% inhibition of IL-2 mRNA in splenocytes isolated from mice repetitively exposed (5 .times. 100 mg/kg over 10 days). Azathioprine (10 mug/mL) inhibited the expression of all mRNAs in rat lymphocyte cultures and inhibited both IL-2 mRNA and actin expression in thymocytes isolated from PVG rats (2 mg/kg/day for 28 days). TBTO (10 nM) induced a 25% inhibition of an early peak of IL-2 receptor mRNA expression within mixed lymphocyte cultures and induced a 10% inhibition of expression of IL-2 receptor mRNA within mixed lymphocyte cultures from rats exposed to 50 mg/kg/day for 28 days. These results demonstrate that appropriate batteries of in vitro tests can identify the four immunomodulatory chems. described here. Observations made in vitro using mol. biol. anal. have been shown to correlate with parallel ex vivo anal.

81
Barratt MD, Basketter DA, Chamberlain M, Payne MP, Admans GD, Langowski JJ. DEVELOPMENT OF AN EXPERT SYSTEM RULEBASE FOR IDENTIFYING CONTACT ALLERGENS. Toxicol in Vitro 1994; 8(4):837-9.

There are currently no in vitro methods for the identification of skin sensitizers (contact allergens). Knowledge relating chem. structure to toxicity can be programmed into expert systems. An historical database contg. results of 294 defined single

substances tested in the guinea pig maximization test to a single protocol was used to derive a set of structural alerts for skin sensitization. Where possible, the approach used was to group the substances according to their most likely mechanism of reaction with skin proteins. Where no mechanism could be identified, structural alerts were derived empirically for groups of mols. with similar chem. functionality. This process has currently resulted in the prodn. of 40 structure-activity rules, which have been incorporated into the expert system DEREK. Rulebases of this type have potential for use as a preliminary screen in toxicol. hazard identification and may ultimately lead to a redn. in the use of lab. animals.

82
Hilton J, Dearman RJ, Debicki RJ, Ramidin LS P, Kimber I. INTERLEUKIN 6 PRODUCTION IN VITRO: AN ALTERNATIVE READ-OUT FOR THE LOCAL LYMPH NODE ASSAY. Toxicol in Vitro 1994;8(4):711-13.

The murine local lymph node assay has been developed as an alternative method for the identification of contact allergens. In contrast to guinea pig tests, which rely on visual assessment of challenge-induced dermal reactions, the local lymph node assay measures events occurring during the induction of skin sensitization. Contact allergic potential is measured as a function of hyperplastic responses in draining lymph nodes following systemic administration of [3H]thymidine.
The authors have now examd. whether the prodn. in vitro of interleukin 6 (IL-6) by draining lymph node cells isolated from sensitized mice provides an alternative endpoint for the local lymph node assay. In comparative expts., the prodn. of IL-6 by lymph node cells in culture correlated closely with proliferative responses in vitro. Only chems. known to cause contact sensitization elicited measurable (>150 pg/mL) IL-6 prodn.; nonsensitizing chems., including skin irritants, did not.
Thus, IL-6 prodn. may provide a useful alternative read-out for the identification of chems. which have a significant skin-sensitizing potential.

83
Jackson EM, Stephens TJ, Rheins LA. ASSESSING HYPOALLERGENIC FACIAL MOISTURIZERS USING IN VIVO AND IN VITRO TESTS. Cosmet Toiletries 1994;109(Jul):83-85. (21 REFS)

To compare in vivo and in vitro test data, 3 commercially available hypoallergenic facial moisturizers underwent in vivo testing using the standard 21-day cumulative irritation test and in vitro testing using a 3-dimensional human skin tissue system. A one-to-one correlation was found with the 24 h readings of interleukin-1-alpha and the in vivo cumulative irritation index.

84
Sauder DN, Shivji GM. KERATINOCYTE CYTOKINE INDUCTION AND ARACHIDONIC ACID RELEASE AN IN VITRO ASSAY FOR ALLERGENICITY OR IRRITANCY. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):175.

No abstract.

85
Gerberich GF. ALTERNATIVE METHODS FOR ALLERGIC CONTACT SENSITIZATION TESTING. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):199.

No abstract.

86
Rambukkana A, Irik D, Bos JD, Kapsenberg ML, Das PK. USE OF HUMAN SKIN ORGAN CULTURE AS AN ALTERNATIVE TO ANIMAL MODELS TO STUDY THE ALLERGENICITY OF LOW MOL. WT. CHEMICAL COMPOUNDS AND HAPTENS. World Congress on Alternatives and Animal use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):200.

No abstract.

LUNG/LUNG TOXICITY
87
Khan MA, Cross FT, Jostes R, Hui E, Morris JE, Brooks AL. MICRONUCLEI INDUCED BY RADON AND ITS PROGENY IN DEEP-LUNG FIBROBLASTS OF RATS IN VIVO AND IN VITRO. Radiation Research 1994;139(1):53-59.

Genotoxic damage induced by radon and its progeny was investigated using the micronucleus assay in deep-lung fibroblasts to compare the response induced in vitro with that induced from inhalation of radon and its progeny in vivo. Male Wistar rats were exposed to 0, 115, 213 and 323 working-level months (WLM) of radon and its progeny by inhalation. After sacrifice, the cells were isolated and grown in culture, and the frequency of micronuclei was determined. A linear increase in the frequency of micronuclei was measured as a function of exposure (micronuclei/1000 binucleated cells = (29 : 9) + (0.47 : 0.04) WLM). To compare exposure in WLM to dose in mGy, and to study how cell proliferation influences the way inhalation

of radon and its progeny induces micronuclei, lung fibroblasts were isolated and exposed in vitro to graded doses from radon and its progeny after either 16 or 96 h in tissue culture. Cell cycle stage at the time of exposure was determined using flow cytometry. Primary lung fibroblasts exposed as either nondividing or dividing cells showed dose-dependent increases in micronuclei (micronuclei/1000 binucleated cells = (33 : 40) : (593 : 68)D and micronuclei/1000 binucleated cells = (27 : 69) : (757 : 88)D, respectively, where D is dose in Gy). Results showed no significant influence (P = 0.20) of cell proliferation at the time of exposure on the frequency of micronuclei induced by radon and its progeny. Comparing dose-response relationships for nondividing cells to the exposure response for cells exposed by inhalation of radon and its progeny, it was estimated that a 1-WLM exposure in vivo caused the same amount of cytogenetic damage as produced by 0.79 mGy in vitro. In vivo/in vitro research using the micronucleus assay in lung fibroblasts serves as a powerful tool to estimate effective dose to cells in the respiratory tract after inhalation of radon and its progeny. Such studies form the basis for understanding the relationship between exposure, dose and biological damage.

88
Fisher RL, Smith MS, Hasal SJ, Hasal KS, Gandolfi AJ, Brendel K. THE USE OF HUMAN LUNG SLICES IN TOXICOLOGY. Human Experimental Toxicol 1994;13(7):466-471.

1. Successful use of agar-filled precision-cut rat lung slices in dynamic organ culture prompted the use of this technology with human lung. 2. The larger tissue mass of a human lung required that the trachea be cannulated with a balloon catheter and subsequently inflated with 4 liters of worm agar/medium mixture and then cooled before being precision-cut into 500 mum thick slices. 3. To characterize the human lung slices, viability and the effects of acrolein and nitrofurantoin were assessed ov a period of 24 h using protein synthesis and nonprotein sulfhydryl content. 4. Control human lung slices synthesized protein at a linear rate and maintained a stable nonprotein sulfhydryl content for 24 h. 5. Slices incubated with acrolein exhibited no significant decrease in protein synthesis or nonprotein sulfhydryl levels until 24 h. 6. incubation with nitrofurantoin exhibited a definite time- and dose-dependent inhibition of protein synthesis, and depletion of the cellular thiol pool. 7. The results indicate that this human lung tissue slice system may be used as an in vitro model to identify and screen pneumotoxicants.

MECHANISMS/TOXICITY
89

Ellouk SA, Jaurand MC. REVIEW OF ANIMAL/IN VITRO DATA ON BIOLOGICAL EFFECTS OF MAN-MADE FIBERS. Environ Health Perspect 1994;102(Suppl 2):47-61. (75 REFS)

This paper reviews the investigations with man-made fibers (MMF). Insulation woods: glasswool (GW), rockwool (RW), slagwool (SW), glass microfibers (GMF), glass filaments (GFiI), and refractory ceramic fibers (RCF) have been used in experimental animals and in in vitro cell systems. A large heterogeneous number of fibers, methods of fiber preparation, size selection, aerosolization, fiber size, and fiber burden measurement were noted, rendering difficult a comparison between results. By inhalation, RCF and asbestos used as positive controls produced a significant tumor increase. In some studies, a low tumor yield was found after inhalation of insulation wools; when all inhalation data were gathered, a significant tumor increase was found with GW. However, it is difficult to draw definitive conclusions on the potential of other fiber types because, in addition to the different compositions of the fibers, differences in fiber number and sizes existed, especially in comparison with asbestos. Moreover, experiments using inoculation, especially by the intraperitoneal route revealed a carcinogenic potential of all fibers types but GFiI and SW. In these two groups a small number of animals has been investigated and the fiber characteristics were sometimes irrelevant. So far, a relationship between the carcinogenic potency and fiber dimensions has been established. Other fiber parameters may be of importance (surface chemistry, biopersistence, fiber structure, for example) but further investigations are necessary to determine the correlations between these parameters and tumor incidence. In vitro experiments have emphasized the fiber characteristics identified in vivo as playing a role in the carcinogenic potency and should be developed as a better approach of the mechanistic effects of MMF.

90
Taylor DL, Debiasio R, Larocca G, Pane D, Post P, Kolega J, Giuliano K, Burton K, Gough B, et al. POTENTIAL OF MACHINE-VISION LIGHT MICROSCOPY IN TOXICOLOGIC PATHOLOGY. Toxicologic Pathol 1994;22(2):145-159.

Major developments in machine-vision light microscopy and in reagent chemistry have led to a renaissance and revolution in the use of the light microscope in biology, biotechnology, and medicine. The potential use of this technology in the field of toxicologic pathology is discussed. It is suggested that a combination of investigating living cells and tissues and fixed samples using the new technologies will lead to understanding mechanisms of toxicity. Examples of the use of the methods in basic cell biology and medicine are presented.

METABOLISM/XENOBIOTICS

91

Randerath K, Zhou GD, Donnelly KC, Safe SH, Randerath E.
DNA DAMAGE INDUCED BY WOOD PRESERVING WASTE EXTRACTS IN VITRO WITHOUT METABOLIC ACTIVATION, AS ASSAYED BY 32P-POSTLABELING.
Cancer Lett 1994;83(1-2):123-8.

Aqueous wood preserving waste (WPW) extracts were tested for their ability to damage DNA in vitro without metabolic activation. Two extracts were prepared from a surface tar and a surface clay soil sample of a WPW site. As assayed by 32P-post-labelling incubation of DNA with these extracts gave rise to highly complex, extract-specific profiles of DNA adducts whose formation depended on the concentration of WPW material. Most of the adducts appeared to be derived from polycyclic aromatic hydrocarbons (PAHs). Three mg organic WPW residue gave Rise to total adduct levels of 13.8 (extract 1) and 66.2 (extract 2) DNA modifications in 10(7) DNA nucleotides, corresponding to 13.9 and 26.9 modifications, respectively, per 10 mg of soil. Thus, extract 2 was more active, although the parent residue had a 1.4-times lower PAH content as determined by gas chromatography/mass spectrometry (GC/MS). DNA adduct formation presumably was a consequence of (i) free radical reactions, possibly involving semiquinones and oxygen free radicals, and (ii) reaction of direct-acting electrophiles, derived from metabolism of WPW toxicants by soil microorganisms. These reactions appeared to be more active in sample 2. The results suggest that ground water at WPW sites contains DNA-reactive compounds posing a cancer hazard to humans. The in vitro DNA adduct assay represents a novel tool to readily assess this type of hazard and the possible effects of remediation measures.

92

Kremers P, Roelandt L, Stouvenakers N, Goffinet G, Thome JP.
EXPRESSION AND INDUCTION OF DRUG-METABOLIZING ENZYMES IN CULTURED

FETAL RAT HEPATOCYTES. Cell Biol Toxicol 1994;10(2):117-25.

An in vitro exptl. model, fetal rat hepatocytes in culture, was metabolically characterized. Several enzymic activities were expressed in these hepatocytes, namely, testosterone hydroxylations. Hepatocytes cultured .ltoreq.3 wk in the presence of dexamethasone and phenobarbital still expressed some drug-metabolizing enzyme activities (e.g., ethoxycoumarin-O-deethylase). The enzymic activities were measured both directly on monolayers during culture and on the corresponding harvested and homogenized cells. The results correlate perfectly with each other. The "on cell" procedure

allows the authors to repeat the assay or to measure several activities on the same cells at different time intervals. The presence of dexamethasone in the culture medium allows the expression and the induction of several cytochrome P 450 isoenzymes, namely, those hydroxylating testosterone. This makes the model particularly attractive for induction expts. as well as for metabolic or toxicol. studies needing longer treatments.

93
Raffali F, Rougier A, Roguet R. MEASUREMENT AND MODULATION OF CYTOCHROME-P450-DEPENDENT ENZYME ACTIVITY IN CULTURED HUMAN KERATINOCYTES. Skin Pharmacol 19994;7(6):345-54.

The metab. of xenobiotics by the epidermis can be studied in vitro by using keratinocyte cultures. We present a simple and rapid method for detg. the activity of cytochrome P 450-dependent enzymes (ethoxycoumarin-O-deethylase, ECOD, and ethoxyresorufin-O-deethylase, EROD) in intact cultured human keratinocytes. Using this method, we studied the effect of a well-known cytochrome P 450 inhibitor (proadifen) and of two inducers (phenobarbital and 3-methylcholanthrene). As reported in vivo, some imidazole compds. (miconazole and econazole) induce ECOD activity at low concns. and inhibit it at high concns. In contrast, two other imidazoles (clotrimazole and sulconazole) only had an inducing effect on ECOD activity. Imidazole itself had no apparent effect on ECOD activity. In conclusion, this model appears to be useful for studying the biotransforming activity of human skin. It allows a rapid evaluation of the inducing/inhibitory effects of xenobiotics.

MODELING SYSTEM/TOXICOLOGICAL
94
Andersen ME. SYNERGY BETWEEN IN VITRO ALTERNATIVES AND PHYSIOLOGICALLY BASED COMPUTER MODELING IMPROVING QUANTITATIVE HEALTH RISK ASSESSMENTS. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):168.

No abstract.

95
Havenaar R, Minekus M, Marteau P, Veenstra J. A COMPUTER CONTROLLED IN VITRO MODEL OF THE GASTROINTESTINAL TRACT OF MONOGASTRIC ANIMALS. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):133.

No abstract.

MUTAGENESIS
96
Muller K, Kasper P, Muller L. LACK OF AFB1-INDUCED MUTAGENICITY IN THE IN VITRO HEPATOCYTE MICRONUCLEUS ASSAY: INVESTIGATION OF POSSIBLE MECHANISMS. Toxicol in Vitro 1994;8(4):533-4.

Several mechanistic expts. were carried out to investigate neg. results obtained with aflatoxin B1 (AFB1) in the in vitro hepatocyte micronucleus assay. The data showed that the lack of AFB1-induced mutagenicity was not due to deficient metabolic activation of the mycotoxin or to an inefficiency of the test protocol. It appeared that the cytotoxic actions of AFB1, specifically the interference with hepatocyte proliferation, prevented the formation of micronuclei.

97
Grant WF. THE PRESENT STATUS OF HIGHER PLANT BIOASSAYS FOR THE DETECTION OF ENVIRONMENTAL MUTAGENS. Mutat Res 1994; 310(2):175-85. (102 REFS)

Higher plants provide valuable genetic assay systems for screening and monitoring environmental pollutants. They are now recognized as excellent indicators of cytogenetic and mutagenic effects of environmental chemicals and are applicable for the detection of environmental mutagens both indoor and outdoor. Comparisons between plant and nonplant genetic assay systems indicate that higher plant genetic assays have a high sensitivity (i.e. few false negatives). Two assays which are considered ideal for in situ monitoring and testing of airborne and aqueous mutagenic agents are the Tradescantia stamen hair assay for mutations and the Tradescantia micronucleus assay for chromosome aberrations. Both assays can be used for in vivo and in vitro testing. Other higher plant genotoxicity assays which have a large number of genetic markers and/or data base and are also highly suitable for testing for genotoxic agents include Arabidopsis thaliana, Allium cepa, Hordeum vulgare, Vicia faba, and Zea mays. Since higher plant systems are now recognized as excellent indicators of the cytotoxic, cytogenetic, and mutagenic effects of environmental chemicals and have unique advantages for in situ monitoring and screening it is recommended that higher plant systems be accepted by regulatory authorities as an alternative first-tier assay system for the detection of possible genetic damage resulting from pollution or the use of environmental chemicals. The results from higher plant genetic assays could make a significant contribution in protecting the public from agents that can cause mutation and cancer. The advantages possessed by higher plant genetic assays, which are

inexpensive and easy to handle, make them ideal for use by scientists in developing countries.

98
Quillardet P, Hofnung M. THE SOS CHROMOTEST: A REVIEW. Mutat Res 1993;297(3):235-279. (179 REFS)

The principles underlying the SOS chromotest were described. The test made use of a sfLA:lacZ operon fusion utilizing Escherichia-coli-PQ37. Induction of sfLA gene by the test agent was manifested as an increase in beta-galactidase activity which is measured by a colorimetric procedure. The data were used to compute a parameter known as the SOS inducing potency (SOSIP). Procedures involved in using the SOS chromotest were considered. Adapting the SOS chromotest to detecting genotoxic chemicals in urine, serum, plasma, fecal extracts, and environmental samples was discussed. Using the SOS chromotest to detect antigenotoxic compounds and the genotoxic effects of radiation was considered. Using the SOS chromotest to identify the types of DNA lesions induced by genotoxic agents was discussed. The possibility of using the SOS chromotest in batteries to measure bioactivation of chemicals was considered. Other assays for detecting genotoxic agents based on genetic fusions were described. The results of evaluations of the ability of the SOS chromotest to detect genotoxic agents based on reviews of the published literature were presented. A total of 751 compounds had been tested in about 40 laboratories. Of these, 404 had detectable genotoxic activity with SOSIPs ranging from about 10(-3) to 10(6). The results obtained for 452 tests could be compared with results obtained in the Ames/Salmonella mutagenicity assay. A total of 373 compounds (82%) produced similar results in both assays, 236 being positive and 137 negative. Most of the discrepancies involved compounds that were very weak SOS inducers and very weak mutagens. When applied to 65 confirmed carcinogens, the SOS chromotest detected 62% of the compounds and the Ames/Salmonella assay detected 77%. For 44 suspected carcinogens, the SOS chromotest detected 66% and the Ames/Salmonella assay detected 68%. The authors conclude that induction of the SOS system as measured in the SOS chromotest appears to be relatively closely correlated with mutagenicity as measured in the Ames/Salmonella assay. The SOS chromotest could be a useful complement to the Ames/Salmonella assay.

99
Blakey DH, Maus KL, Bell R, Bayley J, Douglas GR, Nestmann ER. MUTAGENIC ACTIVITY OF 3 INDUSTRIAL CHEMICALS IN A BATTERY OF IN VITRO AND IN VIVO TESTS. Mutat Res 1994;320(4):273-283. (23 REFS)

The mutagenic activities of three industrial chemicals (propargyl-alcohol (PA), 2-nitroaniline (NA), and 5-methyl-1H-benzotriazole (MBT)) were tested using a battery of in-vitro and in-vivo tests. The test batteries included the Salmonella/mammalian microsome assay (using strains (TA-97), (TA-98), (TA-100), and (TA-102)) with and without S9 activation, the Chinese-hamster ovary (CHO) cell chromosome aberration assay, and the C57BL-mouse bone marrow micronucleus (MNC) assay. Results showed that PA and NA were clastogenic in-vitro, while MBT was not. Both PA and NA induced chromosomal aberrations in CHO cells in-vitro with or without metabolic activation with S9. However, neither induced reverse mutations that were detectable with the Salmonella/mammalian microsome assay. The mouse bone marrow MNC assay showed a slight increase in MNC formation in males but not in females subjected to NA exposure; PA exposure did not result in any such increase. The authors conclude that none of the three tested chemicals are mutagenic in the Salmonella/microsome assay, that only NA is clastogenic in-vivo, and that NA and PA induce chromosomal aberrations in-vitro. They classify MBT as a nonmutagen, PA as an in-vitro mutagen, and NA as an in-vivo mutagen, in conformance with Canadian Health Protection Branch policy.

100
Waters MD, Stack HF, Jackson MA, Bridges BA. HAZARD IDENTIFICATION: EFFICIENCE OF SHORT-TERM TESTS IN IDENTIFYING GERM CELL MUTAGENS AND PUTATIVE NONGENOTOXIC CARCINOGEN. Environmental Health Perspectives, 1993;101(Supplement 3):61-72. (28 REFS)

The efficiency of current procedures for screening mutagens has been examined using a combined data set derived from the Environmental Protection Agency/International Agency for Research on Cancer Genetic Activity Profile EPA/IARC GAP and Gene-Tox databases. Test results were provided from the EPA/IARC GAP and the Gene-Tox Program on substances identified as mammalian germ cell mutagens. The review indicated that the sensitivity of the battery of in-vitro short term mutagenicity tests currently used to identify potential germ cell mutagens was about 90%. The authors note that some possible improvements in the deployment of such tests, such as the inclusion of a mammalian assay for induction of aneuploidy, are worthy of further study. The data presented affirms the usefulness of short term tests in hazard identification and support current regulatory testing strategies.

101
Uno Y, Takasawa H, Miyagawa M, Inoue Y, Murata T, Yoshikawa K. AN IN VIVO-IN VITRO REPLICATIVE DNA SYNTHESIS (RDS) TEST USING RAT HEPATOCYTES AS AN EARLY PREDICTION ASSAY FOR NONGENOTOXIC

HEPATOCARCINOGENS: SCREENING OF 22 KNOWN POSITIVES AND 25 NONCARCINOGENS. Mutat Res 1994;320(3):189-205. (48 REFS)

A comparison of replicative DNA synthesis (RDS) test findings was made with 22 nongenotoxic (Ames negative) putative rat hepatocarcinogens and 25 noncarcinogens. Test chemicals were administered to male F344-rats by single oral gavage or subcutaneous injection. Test chemicals included acetaminophen, 11-aminoundecanoic-acid, carbon-tetrachloride, chlorendic-acid, chloroform, clofibrate, p,p'-DDT, dehydroepiandrosterone, di(2-ethylhexyl)phthalate, diethylstilbestrol, 1,4-dioxane, D,L-ethionine, 17-alpha-ethynylestradiol, Alpha-hexachlorocyclo-hexane, methyl-carbamate, phenobarbital-sodium, polybrominated biphenyls, safrole, tannic-acid, thioacetamide, urethane, and Wy-14,643. Simple acute toxicity was tested, the maximum tolerated dose of each sample was set, and time/course experiments were performed. Primary hepatocyte cultures were prepared and assessment of RDS induction was performed. Compared to controls, the RDS test gave positive results for 18 hepatocarcinogens and negative results for 20 noncarcinogens. The authors conclude that the in-vivo/in-vitro RDS test using rat livers is a reliable screening system which is able to predict the carcinogenicity of Ames negative putative rat liver carcinogens with an 81% concordance with long term in-vivo results.

102
Madle S. SERIES CURRENT ISSUES IN MUTAGENESIS AND CARCINOGENESIS NO. 47 STRATEGIES AND PHILOSOPHIES OF GENOTOXICITY TESTING HIGHLY SOPHISTICATED VERSUS PRAGMATIC REGULATORY APPROACHES REPLY TO ZEIGER 1994 STRATEGIES AND PHILOSOPHIES OF GENOTOXICITY TESTING WHAT IS THE QUESTION? Mutat Res 1994;308(1):111-112.

No abstract.

103
Wiencke JK, Spitz MR. IN VITRO CHROMOSOMAL ASSAYS OF MUTAGEN SENSITIVITY AND HUMAN CANCER RISK. Cancer Bulletin (Houston) 1994;46(3):238-246.

No abstract.

NEPHROTOXICITY
104
Ruegg CE. PREPARATION OF PRECISION-CUT RENAL SLICES AND RENAL PROXIMAL TUBULAR FRAGMENTS FOR EVALUATING SEGMENT-SPECIFIC NEPHROTOXICITY. J Pharmacol Toxicol Methods 1994;31(3):125-33. (31 REFS)

Previous research in animals and humans has demonstrated that many nephrotoxic chems. induce selective injury within the kidney affecting either renal proximal straight (PST) or proximal convoluted (PCT) tubules. Selective injury has also been obsd. following in vitro nephrotoxicant exposure to precision-cut renal slices and isolated PCT and PST segments. These in vitro models provide a means of comparing and contrasting basic mechanistic differences which render these segments innately susceptible to nephrotoxicant injury. In this article, methods for prepg. precision-cut slices and isolating PST and PCT segments will be reviewed.

105
Gilbert T, Gaonach S, Moreau E, Merlet-Benichou C. DEFECT OF NEPHROGENESIS INDUCED BY GENTAMICIN IN RAT METANEPHRIC ORGAN CULTURE. Lab Invest 1994;70(5):656-66.

In the rat, in utero exposure to gentamicin during early renal differentiation leads to a permanent nephron deficit. The aim of the present study was to analyze, in vitro, the potential direct effect of gentamicin on early nephrogenesis. We used paired rat metanephric organ cultures from 14 (F14) or 15-day-old (F15) fetuses. We measured gentamicin accumulation into explanted metanephroi and then assessed in vitro growth in the absence or presence of the drug. Glomerular labeling and counting were performed on the whole explant to analyze the effect of antibiotics on early nephrogenesis. Growth of F14 metanephric explants in the presence of 50 mug of gentamicin/mL was significantly reduced from 4 days onwards as compared to controls, whereas F15 explants grown with gentamicin displayed a normal in vitro development. After 6 days of culture, F14 and F15 explants had the same accumulation of gentamicin (1 mug/mg protein) but the gentamicin content was 4 times larger in F15 explants. At both ages, gentamicin-exposed metanephric explants exhibited a significant redn. in their no. of nephrons. However, the effects of 50 mug of gentamicin/mL on nephrogenesis were significantly more drastic on F14 than F15 explants (35% vs. 18%). When grown with 0.5 mug of gentamicin/mL, F14 explants still exhibited a 16% defect in nephrogenesis as compared with controls, and about the same redn. was obsd. for cultures in the presence of 100 mug/mL of streptomycin and 100 IU/mL of penicillin. Incubation of F14 explants with streptomycin alone for 6 days had no effect on nephrogenesis. These results indicate that gentamicin induces a significant redn. in the no. of nephrons in metanephric explants and that this effect is more important on less differentiated metanephroi. Metanephric organ culture combined with glomerular labeling represents a useful model to test the effect of various growth factors and other drugs on early nephrogenesis.

106
Prodanchuk NG, Sinchenko VG, Vlasyk LI. EVALUATION OF NEPHROTOXICITY IN IN VITRO AND IN VIVO. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994; 7(2):147.

No abstract.

NEUROTOXICITY
107
Purcell WM, Atterwill CK. HUMAN PLACENTAL MAST CELLS AS AN IN VITRO MODEL SYSTEM IN ASPECTS OF NEURO-IMMUNOTOXICITY TESTING. Human Experimental Toxicol 1994;13(6):429-433.

In both the developing and adult nervous systems, nerve growth factor (NGF) influences neuronal survival, differentiation and recovery following insult. The effect of NGF upon human placental most cells (HPMC) was investigated, since it is known that rodent most cells express a functional receptor for NGF and secrete histamine upon challenge with this neurotrophic factor. Furthermore, human placental tissue contains a significant amount of NGF and expresses a NGF receptor. HPMC were shown to secrete histamine in a concentration dependent manner in response to NGF (0.001-10.0 mug ml-1) in the presence of the lipid cofactor phosphatidylserine (10.0 mug ml-1). NGF induced histamine release from isolated HPMC with an EC50 of 0.1 mug ml-1 NGF and maximal secretion of total cellular histamine of 22.3 : 3.4% at 3.0 mug ml-1. The response was shown to be a secretory process, dependent upon the presence of exogenous calcium ions and to be pH- and temperature-sensitive. HPMC are suggested to be a suitable primary cell model for use in aspects of in vitro toxicity testing, in terms of assessing the neuro-immunotoxic potential of neurotrophic therapeutics, In addition, mechanistic studies concerning those xenobiotics which may exert their neurotoxic effect via interaction with neurotrophic factors and, or their receptors, may be studied in this human cell model.

108
Cookson MR, Pentreath VW. ALTERATIONS IN THE GLIAL FIBRILLARY ACIDIC PROTEIN CONTENT OF PRIMARY ASTROCYTE CULTURES FOR EVALUATION OF GLIAL CELL TOXICITY. Toxicol In Vitro 1994; 8(3):351-359.

Astrocyte-enriched primary glial cell cultures from the rat cerebral cortex were exposed to a range of neurotoxic compounds and the effects on three proteins were examined by enzyme-linked

immunosorbent assay (ELISA). The most consistent marker for astrocyte toxicity was the intermediate filament protein glial fibrillary acidic protein (GFAp). Many toxicants reported to have specificity for astrocytes (e.g. mercuric chloride, aluminium chloride, toluene or ethanol) produced a similar dose-response curve: increases in the GFAp content of the cells at sub-cytotoxic concentrations with attenuation at higher concentrations. Some of the concentrations required to increase GFAp were extremely low, indicating that this is a sensitive and consistent marker of cellular damage, as has been shown in vivo. Toxicants that have neuronal specificity (such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridinium or acrylamide) had no effects on GFAp levels, indicating that the system measures the susceptibility of astrocytes to specific toxicants rather than neurotoxicity. The levels of S-100 protein and vimentin were measured for a smaller number of toxicants. S100 was a less sensitive and inconsistent marker in comparison with GFAp, whilst vimentin levels were not seen to increase with any tested compound. These results suggest that astrocytes have a valuable place in culture models for neurotoxicity testing.

109
Campbell IC, Abdulla EM. THE EFFECT OF REPRESENTATIVE NEUROTOXIC CHEMICALS ON THE LEVEL OF NEUROFILAMENT PROTEIN SUBUNITS IN NEURONAL CELL LINES AN IN VITRO MEASURE OF NEUROTOXICITY? World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):188.

No abstract.

110
Johnson AR, Cook GM W, Keynes RJ. IN VITRO ASSAYS FOR MOLECULES THAT INHIBIT GROWTH CONE MOTILITY DURING NEURAL DEVELOPMENT AND REGENERATION. NeuroProtocols 1994;4(2):121-8.

There is increasing evidence that mols. that inhibit growth cone motility are involved in the guidance of axons to their appropriate targets during neural development and contribute to the suppression of axon regeneration in the mammalian CNS. Two tissue culture phenomena have been used to detect and monitor these mols.: inhibition of neurite outgrowth and growth cone collapse. In neurite outgrowth assays the inhibitory material is used as a culture substratum. It can be presented to neurons either as a continuous layer or in a form that growing axons will encounter, such as an explant or a stripe. Tissue explants or sections, monolayer cultures of cells, membrane fractions, and purified or partially purified material have all been used. In the growth cone collapse assay, the growth cones of axons

extending on a permissive substratum are treated with liposomes incorporating the putative inhibitory material. This method is particularly useful for testing the inhibitory effects of membrane-derived mols. The relevance of results obtained with these in vitro assays to axon growth phenomena in vivo must always be established. Their principal value lies in the provision of a means of monitoring biochem. purifn. procedures aimed at identifying and characterizing mols. that inhibit nerve growth.

111
Ehrich M, Correll L, Veronesi B. NEUROPATHY TARGET ESTERASE INHIBITION BY ORGANOPHOSPHORUS ESTERS IN HUMAN NEUROBLASTOMA CELLS. Neurotoxicology (Little Rock) 1994;15(2):309-314.

Certain organophosphorus compounds (OPs) produce a delayed neuropathy (OPIDN) in man and some animal species. Capability to cause OPIDN is generally predicted in animal models by early and irreversible inhibition of neuropathy target esterase (NTE, neurotoxic esterase). In this study NTE inhibition in response to OP exposure was examined in cell culture, using the human SH-SY5Y neuroblastoma cell line. Cells were exposed for 1 hr to equimolar (1 of 6 OPs associated with OPIDN in vivo (including 2 protoxicants and 4 active (-P = O) toxicants), and 8 OPs that do not produce delayed neuropathy in animal models (including 5 protoxicants and 3-P=O compounds). The -P=O compounds that cause OPIDN in animal models inhibited NTE > 60% at the test concentration; -P = O compounds that do not cause OPIDN in animal models inhibited NTE < 30%. Protoxicants did not inhibit NTE at the test concentration, reflecting their limited metabolism in the human cell line. These results indicate that human neuroblastoma cells have potential use in the initial screening of bioactive OPs with capability for causing OPIDN.

112
Manthorpe M, Muir D, Pettmann B, Varon S. DETECTION AND ANALYSIS OF GROWTH FACTORS AFFECTING NEURAL CELLS. Neuromethods 1992; 23(Iss Practical Cell Culture Techniques):87-137. (120 REFS)

No abstract.

113
Bonheur JL, Laev H, Vorwerk C, Karpiak SE. TRAUMATIC INJURY OF SPINAL CORD CELLS IN VITRO REDUCED BY GM1 GANGLIOSIDE. Restor Neurol Neurosci, 1994;6(2):127-33.

GM1 ganglioside (monosialoganglioside) is a significant endogenous component of central nervous system (CNS) cellular

membranes, thereby contributing to the membranes' integrity and function. Exogenous gangliosides have been shown to be incorporated into plasma membranes and exert neuroprotective effects on damaged neuronal tissue(s). An in vitro method of phys. injury (trauma) previously described which used cultures derived from fetal mouse spinal cord [38] was adapted for these studies in order for the authors to assess GM1's neuroprotective efficacy. Injury was induced by uniformly crosshatching the spinal cell cultures with a 1 mm plastic pipet tip. The extent of injury and the effects of GM1 ganglioside posttreatment (80 muM) was assessed after 48 h by measuring lactate dehydrogenase (LDH) released and by observing changes in the plasma membrane surface distribution of endogenous GM1 using chole ra toxin/antitoxin/fluorescent antibody immunohistochem. A gradient of injury, from the zone of max. injury to partially traumatized or non-injured areas, was seen using immunohistochem. The primary injury zone in this gradient was characterized by areas of swollen or dead cells and abnormal or degenerating cell processes. At further distances, cells were obsd. to be nearly normal, with intact fibers. This gradient of injury may reflect proximate (at the locus of trauma) and distant effects of the release of neurotoxic levels of endogenous glutamate (Glu) and other excitatory amino acids. Ganglioside GM1 treatment resulted in a significantly reduced (>75%) release of LDH as well as enhanced cell and process integrity indicative of reduced tissue injury. These initial results indicate that GM1's previously documented neuroprotective effects using neuronal culture systems can be generalized to injured spinal cells in vitro wherein there is evidence for preservation (rescue) of cellular plasma membranes after injury as reflected by reduced cell loss, swelling, and process degeneration, as well as decreased LDH release.

114
McLane JA. COMPARISON OF IN VITRO AND IN VITRO MODELS OF PERIPHERAL NEUROPATHY INDUCED BY TOXINS AND THERAPEUTIC AGENTS. Veterans Administration/Research and Development (15), 810 Vermont Ave. N.W., Washington, D.C. 20420, United States of America. FEDRIP Database. National Technical Information Service(NTIS), Springfield, VA; 1994.

Objectives: Axonal transport is a critical cellular process involved in the development and maintenance of neurones. Traditionally, this process has been studied in in vivo animal models. We propose to develop methods which will allow for the detailed study of axonal transport using video-microscopy techniques on cultured neurite-producing cells. We hypothesize that these methods will allow us to obtain information regarding the neurotoxicity of drugs and chemicals, and the mechanism(s) by which neurotoxins produce peripheral neuropathy. Research Plan:

In the proposed studies we will compare the effects of known neurotoxins (i.e., acrylamide, 2,5-hexanedione, p-bromophenylacetylurea) on fast axonal organelle transport in sciatic nerve axons from animals and in neuroblastoma cell neurites. Methods: The animal experiments will be carried out with male, viral-antibody-free Sprague-Dawley rats which are eight weeks old at initiation of toxin treatment. Animals will be administered doses of the test toxins which have previously been shown to produce peripheral neuropathy. The development of the neuropathy will be followed using standard electrophysiologic measures of conduction velocities and peak amplitudes. When the neuropathy has developed fast axonal transport will be studied using computer-enhanced video-microscopy. The cell culture experiments will make use of Neuro2a cells, a mouse neuroblastoma cell line. Concentrations of test toxins will be identified which lead to loss of cell neurites without immediate death of the cell. Fast organelle transport will be examined in the neurites of toxin treated cells after different lengths of toxin exposure. The toxin effects on axonal transport will then be compared in the in vivo and in vitro models. Findings: We have found that a short exposure (1-3 hours) of the Neuro2a cells to 2 mM acrylamide produces a loss of neurites over the next two days. We are now determining the percent recovery of cells and timing of recovery following removal of the toxin. We are developing the methodologies for video-microscopy here at Hines. Clinical Relevance: In vitro cell cultures are already utilized widely to screen for cytotoxicity of chemicals and to test whether chemicals cause cell transformation as a prelude to the development of cancer. If it is found that toxins alter organelle transport in the in vivo and in vitro models similarly, we suggest that neuron-like cell cultures have a valuable potential in the study of neuropathogenic mechanisms and in screening chemicals, new drugs or environmental agents for neuropathological activity. Single cell models would not only reduce the number of animals needed in neurotoxicological and neuropathological studies, but will also be more economical in terms of experimental time, space, and resources.

115
Wormser U, Kritzler JS, Eizen O, Reibstein I. IN VITRO BIOASSAY FOR DETECTION AND MONITORING EXPOSURE TO ENVIRONMENTAL NEUROTOXINS. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):178.

No abstract.

OCULAR TOXICITY

116
Vinardell MP, Macian M. COMPARATIVE STUDY OF THE HET-CAM TEST AND THE DRAIZE EYE TEST FOR ASSESSMENT OF IRRITANCY POTENTIAL. Toxicol In Vitro 1994;8(3):467-470.

The ocular irritancy potential of different substances used as vehicles has been tested by the Draize test method and the alternative hen's egg test-chorioallantoic membrane (HET-CAM) test. With the latter test, carboxymethylcellulose was not found to be irritant and can be used to suspend insoluble substances to be assessed by this method. The ocular irritancy potential of six commercial disinfectants was also tested by the Draize test method and the alternative HET-CAM test. Products were assigned to categories of irritancy, according to two different sets of criteria for the Draize test results, and according to irritancy potential assessed by the HET-CAM test. When both sets of results for Draize categories were compared with those for the HET-CAM test, four of the six products tested showed similar irritancy potential. The remaining two products gave false positive results on the HET-CAM compared with the Draize test.

117
Earl LK, Jones PA, Dixit MB, O'Brien K AF. COMPARISON OF FIVE POTENTIAL METHODS FOR ASSESSING OCULAR IRRITATION IN VITRO. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):145.

No abstract.

118
Heinze J, Spence ET, Rachui SR, Duke MA, Robertson WD. PREDICTING OCULAR IRRITATION POTENTIAL OF SURFACTANT IN VITRO USING THE SKIN-2T-M MODEL ZK1200. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):192.

No abstract.

119
Doyle JM, Dressler WE, Spence ET, Rahui S. UTILITY OF TWO IN VITRO OCULAR IRRITATION MODELS THE BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY BCOP AND THE SKIN-2T-M MODEL ZK1200 IN CATEGORIZING AND RANKING THE RELATIVE OCULAR IRRITANCY POTENTIAL OF CHEMICALS AND PERSONAL CARE PRODUCTS. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994; 7(2):191.

No abstract.

120
Rougier A, Cottin M, De Silva O, Catroux P, Roguet R, Dossou KG.
THE USE OF IN VITRO METHODS IN THE OCULAR IRRITATION ASSESSMENT OF COSMETIC PRODUCTS. Toxicol in Vitro 1994;8(4):893-905.

The ocular tissue is a complex system consisting of corneal and conjunctival epithelial cells, the underlying corneal stroma and assocd. endothelial cells. Exposure to chems. may result in responses ranging from mild, slight redness and itching, to severe injury with loss of corneal epithelium, damage to stroma, inflammatory infiltration and loss of vision. This complexity hinders the development of in vitro methods able to replace

animal testing. Various in vitro techniques have been proposed and subsequently developed as potential replacements for ocular toxicity screening on animals. Over the past 2 yr, eight methods have been evaluated in these labs. The endpoint of these methods could be linked to one or to several clin. events occurring in the in vivo eye irritancy process described above. Using these systems, a battery of four complementary in vitro assays has been developed. For the categories of ingredients and cosmetic products investigated , the promising results obtained suggest that in vitro methods of ocular risk assessment may be used increasingly in the future.

121
Martin SA, Roy TA, Saladdin KA, Fleming BA, Mackerer CR. SAFETY EVALUATION OF PETROLEUM PRODUCTS USING AN IN VITRO EYE IRRITATION TEST BATTERY. Toxicol in Vitro 19994;8(4):715-17.

An in vitro eye irritation test battery (IVEye) composed of the EYTEX and modified agarose diffusion method (MADM) assays was evaluated for use as a predictive, economical screen and/or adjunct for the Draize eye test. EYTEX mimics corneal opacification using a synthetic matrix of proteins that is intended to produce measurable opacity on exposure to chem. irritants in proportion to their ocular irritation potential. MADM is a cytotoxicity-based assay consisting of NCTC clone 929 mouse fibroblasts overlayed with 1% agarose in culture medium. Potential eye irritation is measured macroscopically as the area of decolorization (neutral red release) around the area of chem. application and microscopically as the percentage of cell lysis resulting from chem. application. Of the 70 materials tested in the IVEye for which Driaze eye test data also exist, the battery correctly identified 38 materials as nonirritants and 30 as irritants, with 2 false pos. and no false negs. Nonparametric

anal. of the data show the battery to have a sensitivity of 100%, a specificity of 95% and a predictive value of 94%. The irritation class correlation (equivalence; irritation ranking) between EYTEX alone and the Draize data was 85%. These data support the use of IVEye as an accurate, reproducible and cost-effective in vitro method for identifying the eye irritation potential of petroleum products.

122
Roy TA, Saladdin KA, Mackerer CR. EVALUATION OF THE EYTEX SYSTEM AS A SCREEN FOR EYE IRRITANCY OF PETROLEUM PRODUCTS. Toxicol in Vitro 1994;8(4):197-8.

Eighty-nine petroleum products were tested in the EYTEX in vitro assay and the results were compared with data from Draize tests on the same materials. All 26 chems. assessed as irritant in the Draize test were classified as irritant by the EYTEX assay; 61 of the 63 non-irritants were correctly classified by the in vitro assay. The correlation between the results from the EYTEX system and the Draize data was 89%, and the predictive value of the EYTEX system (percentage of EYTEX irritants that are true irritants) was 93%. When the same chems. were tested in another lab., the coeff. of detn. for the comparison of the EYTEX scores from the two labs. was R2 = 0.86. The results demonstrate the reliability and interpretability of the EYTEX assay carried out in our lab. and therefore the viability, if not the validity, of this in vitro method as a screening test for the Draize assay.

123
Sina JF. VALIDATION OF THE BOVINE CORNEAL OPACITY-PERMEABILITY ASSAY AS A PREDICTOR OF OCULAR IRRITATION POTENTIAL. In Vitro Toxicol 1994;7(3):283-90.

The author's task was to develop an approach to assessing ocular irritation, with minimal use of animals, so that it could be applied inhouse for worker safety testing. The centerpiece of the multifaceted approach was the bovine corneal opacity and permeability (BCOP) assay, a test that measures opacity and cell sheet integrity in a target tissue: the cornea. Initial studies indicated that the BCOP gave an overall concordance with in vivo data of 85%. Two specific findings were that although suspensions could be tested, hydroscopic compds. tended to float on the exposure medium and adequate exposures could not be obtained; and that some compds. caused irritation in animals only after a delay of 24-72 h, and these could not be detected in vitro. The BCOP method proved to be easily transferable, with good reproducibility in a small interlab. study. Overall concordance was approx. 83% among the six participants. Some slight modifications were made to the assay based on the results of this

study, and a larger interlab. study was begun in order to broaden further experience and to attempt to define other areas of potential difficulty. Twelve labs. participated in evaluating 52 diverse chems., and interlab. variability was quite low. Concordance was approx. 80%, with the false-neg. and false-pos. rate dependent upon the in vivo classification system generally in use at a particular lab. In a comparison study in which 36 pharmaceutical manufg. intermediates from 11 companies were all tested in the BCOP and 6 other assays, the BCOP was found to be among the most accurate tests. A tiered approach, combining results from other assays with the BCOP data, did not appear to provide an enhanced predictive value. Based on this body of data, it was considered that the BCOP assay is valid for the specific purposes and for the types of chems. included in the studies (i.e., mainly pharmaceutical intermediates and raw materials).

124
Noever DA, Matsos HC, Cronise RJ, Looger LL, Relwani RA. COMPUTERIZED IN VITRO TEST FOR CHEMICAL TOXICITY BASED ON TETRAHYMENA SWIMMING PATTERNS. Chemosphere 1994;29(6):1373-84.

An app. and a method for rapidly detg. chem. toxicity have been evaluated as an alternative to the rabbit eye irritancy test (Draize). The toxicity monitor includes an automated scoring of how motile biol. cells (Tetrahymena pyriformis) slow down or otherwise hang their swimming patterns in a hostile chem. environment. The method, called the Motility Assay (MA), is tested for 30 s to det. the chem. toxicity in 20 aq. samples contg. trace org. and salts. With equal or better detection limits, results compare favorably to in vivo animal tests of eye irritancy.

125
Lewis RW, McCall JC, Botham PA. DEVELOPMENT OF AN IN VITRO TEST BATTERY FOR USE WITHIN A STEPWISE APPROACH TO THE ASSESSMENT OF OCULAR IRRITANCY IN VIVO. Toxicol in Vitro 1994;8(4):865-6.

The current OECD guideline for the assessment of eye irritation recommends, within its initial considerations, the use of data fro skin irritation tests as a pre-screen to detect the most severely irritating materials, its being assumed that materials that are severely irritating to the skin are also significantly irritating to the eyes. However, anal. of data for 223 materials, tested in this lab. for both dermal and ocular irritancy, revealed that only 23% of severe eye irritants were also severe skin irritants. This resulted in a significant no. of rabbits developing severe ocular effects that had not been predicted from the dermal responses. This study reports

the results of an alternative approach for predicting severe eye irritants. The approach was a two-stage in vitro test battery; the first stage was a cytotoxicity assay using the K562 cell lines; the second was the isolated rabbit eye test. In contrast to the use of skin irritation tests, the in vitro battery was significantly more predictive (83% of severe eye irritants were detected). Although the incidence of false pos. responses in the assay precludes its routine use as a replacement for the in vivo rabbit eye test, the test battery provides a powerful aid to reducing animal use and guiding in vivo studies to minimize the severity of effects.

126
Cottin M, Dossou KG, De Silva O, Tolle M, Roguet R, Cohen C, Catroux P, Delabarre I, Sicard C, Rougier A. RELEVANCE AND RELIABILITY OF IN VITRO METHODS IN OCULAR SAFETY ASSESSMENT. In Vitro Toxicol 1994;7(3):277-82.

The authors have concd. their efforts for many years to select, among various so-called alternative methods, several in vitro tests reliable for the evaluation of ocular irritation of cosmetics without using animals. Eye irritation is a multistep process including different mechanisms that cannot be investigated by one in vitro test alone. It is thus essential to use several complementary tests to evaluate eye irritation correctly. Moreover, some in vitro methods exhibiting tech. limitations are not suitable for the study of complex mixts. such as creams, mascaras, etc. The authors thus decided to use numerous tests and to make successive studies of various categories of cosmetics through the tests that were adapted. Thereafter different statistical studies were conducted, one of which (multivariate anal.) was able to show which methods were redundant and which were complementary. The effects of around 90 products with historical Draize data were evaluated on various alternative systems (including protein mixt., cells, bacteria, reconstructed skin equiv., isolated cornea, hen's egg test at the chorioallantoic membrane [Het-CAM]). The results allowed to select different combinations of tests according to the category of cosmetics.

127
Chetoni P, Bianchi LM, Torracca MT, Saettone MF, Conte U. PREPARATION AND IN VITRO/IN VIVO EVALUATION OF OPHTHALMIC MINIMATRIXES CONTAINING TIMOLOL. Boll Chim Farm 1993; 132(10):398-9.

Ophthalmic prepns. of timolol maleate were prepd. by direct compression with known excipient. In std. release tests in vitro, polymer coated minimatrixes release the drug with 0-order

kinetics for 30 h; in rabbits, the prepns. produced no ocular irritation and gave drug concns. of 0.1 mug/muL in the lachrymal fluid 26 h after application.

128
Gordon VC, Latta C, Palaysi A, Dyrness K, Pu G, Kim Heidi. EVALUATION OF A SYSTEM FOR ASSESSING OCULAR TOXICITY OF SURFACTANTS AND SURFACTANT-BASED FORMULATIONS. Comun Jorn Com Esp Deterg 1994;25:533-45.

The EYTEX System is an in vitro method to predict in vivo ocular irritation using changes of relevant macromols. upon exposure to chem. and formulations to predict in vivo irritancy and toxicity endpoints. The method has been extensively evaluated using chem. and formulations from diverse classes with differing ranges of toxicity, representing different mechanisms of ocular toxicity. Results using the in vitro test correlate broadly to the Draize in vivo test results. Quantification of the EYTEX Method is based on the measurement of the changes in optical d. of the matrix which are sensitive to the conformation, hydration of protein, as well as the order of the matrix. These changes, which occur when the matrix is exposed to chem. and formulations can be used to predict in vivo ocular irritancy. Calibrators and controls provide assay standardization with a 4-8% coeff. of variation. The evaluation presented here was designed to test surfactants and surfactant formulations in the EYTEX System. Using a modified protocol within creased activation of the matrix (lower pH) and decreased incubation time (5 h vs 24 h), the Rapid Membrane Assay (RMA), was evaluated using 18 surfactants and 36 surfactant formulations. Surfactants were tested at multiple concns. and a highest qualified concn. (HQC) detd. At the HQC, the EYTEX Draize Equiv. (EDE) scores were calcd. form a calibration curve. Correlations of the in vivo ocular irritation data with the EDE were as follows: Ar = 0.73 for the 18 surfactants at 25 different concns. and r = 0.78 for the 14 shampoo formulations. For 22 other surfactant formulations, the sensitivity id 93% and the specificity is 75%. The addn. of an anti-irritant to an irritant anionic surfactant showed a decrease initiation both in vivo and in vitro. This study demonstrates the ability, reproducibility and relevance to in vivo ocular irritation data of the EYTEX Method in the evaluation of surfactants and surfactant-based products.

129
Pierard GE, Goffin V, Pierard-Franchimont C. CORNEOSURFAMETRY: A PREDICTIVE ASSESSMENT OF THE INTERACTION OF PERSONAL-CARE CLEANSING PRODUCTS WITH HUMAN STRATUM CORNEUM. Dermatol 1994; 189(2):152-6.

Corneosurfametry is introduced as a noninvasive quantitative test rating the interaction between surfactants and human stratum corneum. It may be used as a predictive irritancy test. BACKGROUND: Surfactants present in personal-care products elicit multiple effects on the stratum corneum. With upcoming regulations avoiding animal experiments and ethical considerations for human testing, there is a need for new in vitro methods evaluating irritancy. DESIGN: Corneosurfametry entails collection of cyanoacrylate skin surface strippings and short contact time with surfactants followed by staining samples with toluidine blue and basic fuchsin dyes. Measurements are made by reading the color of samples using reflectance colorimetry. RESULTS: The intensity of color increases with irritancy potential of the surfactant. Results are reproducible, and great differences are noted among a series of diluted shampoos, shower gels and facial cleansing gels. CONCLUSION: Corneosurfametry is proposed as a rapid in vitro method allowing a predictive grading of surfactants related to irritancy.

130
Grant RL, Acosta D. COMPARATIVE TOXICITY OF TETRACAINE, PROPARACAINE AND COCAINE EVALUATED WITH PRIMARY CULTURES OF RABBIT CORNEAL EPITHELIAL CELLS. Exp Eye Res 1994;58(4):469-78.

Cocaine was first used as a topical anesthetic for the eye by Dr Carl Koller in 1884. It became evident that this agent produced erosion of the corneal epithelium in high doses or with repeated use. Synthetic local anesthetics such as tetracaine and proparacaine were developed which were more potent and less toxic than cocaine, but still produced corneal epithelium defects if used chronically. This investigation was undertaken to compare and rank the cytotoxicity of the most commonly used ocular local anesthetics, tetracaine, proparacaine and cocaine, with primary cultures of rabbit corneal epithelial cells. Cultures were exposed to either low concentrations of local anesthetics for 4-24 hr or to higher concentrations of local anesthetics for 15-120 min. Plasma membrane integrity was evaluated by measuring leakage of the cytosolic enzyme, lactate dehydrogenase, into the

medium. Cell shape changes were evaluated by observing morphological changes. Mitochondrial dehydrogenase activity and cell viability were assessed by measuring 3-(4,5-dimethythiazol-2yl)-2,5-diphenyl tetrazolium bromide reduction. The cytotoxicity of the local anesthetics as evaluated by the lactate dehydrogenase leakage cytotoxicity test was ranked as follows: tetracaine (EC50 = 0.96 mM) >> proparacaine (EC50 = 4.4 mM) > cocaine (EC50 = 9.7 mM). The mitochondrial reduction assay seemed to be more sensitive than the lactate dehydrogenase leakage test in predicting toxicity: tetracaine (EC50 = 0.81 mM) >> proparacaine (EC50 = 3.4 mM) > cocaine (EC50 = 7.1 mM). When

corneal epithelial cells were treated with local anesthetics, marked morphological changes occurred at concentrations that did not cause a decrease in viability. This was especially true for cocaine-treated cells. Tetracaine and proparacaine have the same anesthetic potency in vivo, although tetracaine is considered to be more irritating than proparacaine. This in vitro study showed that tetracaine was approximately four times more toxic than proparacaine. Cocaine was less toxic in vitro than proparacaine and tetracaine when compared on an equimolar basis, but in vivo it may be more toxic because of the higher concentrations that must be used to obtain the same degree of anesthesia as well as its marked effects on cell morphology.

131
Martin KM, Bernhofer LP, Stott CW. PRELIMINARY EVALUATION OF A THREE DIMENSIONAL CORNEAL CONSTRUCT AS AN IN VITRO MODEL FOR OCULAR IRRITATION. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):164.

No abstract.

132
Reece B, Rozen M, Long D, Bryan B. ASSESSMENT OF THE OCULAR IRRITANCY POTENTIAL OF SURFACTANTS USING SEVERAL DIFFERENT IN VITRO SYSTEMS. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):163.

No abstract.

133
Roberts DA, Perkins MA, Wallace K, Mun G, Curren R. INTERLABORATORY VALIDATION OF A NEW IN VITRO TISSUE EQUIVALENT ASSAY TEA FOR EYE IRRITATION ASSESSMENTS. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994; 7(2):191.

No abstract.

134
Katsnelson BA, Privalova LI, Sharapova NE, Kislitsina NS. SIGNIFICANCE OF SHORT-TERM CYTOTOXICITY TESTS FOR HYGEINIC ASSESSMENT OF INDUSTRIAL AEROSOLS. Med Tr Prom Ekol 1993;1:2-7. (14 REFS)

The evaluation of low-soluble particles toxicity for human

phagocytes (primarily, for macrophages) was proved to have advantages in predicting their effects on the human body and in rapid toxico-hygienic regulation. The preference is given to tests "in vitro". If comparative toxicities of substances in group are discordant according to different "in vitro" tests the decision must be made after the "in vivo" evaluation of cytotoxicity: in 24 hours after the intratracheal administration of small doses of these low-soluble particles the bronchoalveolar lavage is examined cytologically. Reliable neutrophilia in lavage is of great value.

135
Swanson JE, Edwards SM, Donnelly TA, Lake LK, Osimitz TG, Rheins LA. PREDICTING OCULAR IRRITANCY OF FULL-STRENGTH CLEANERS AND STRIPPERS USING THE TISSUE EQUIVALENT ASSAY TEA. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994; 7(2):190.

No abstract.

136
Kahn CR, Walker TL. HUMAN CORNEAL EPITHELIAL PRIMARY CULTURES AND CELL LINES WITH EXTENDED LIFESPAN CAN BE USED TO STUDY MECHANISMS OF CORNEAL INJURY AND SUBSEQUENT REPAIR. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994; 7(2):124.

No abstract.

137
Cottin M, Rougier A, De Silva O, Roguet R, Cohen C, Catroux P, Delabarre I, Sicard C. RELEVANCE AND RELIABILITY OF IN VITRO METHODS IN OCULAR SAFETY ASSESSMENT. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994; 7(2):132.

No abstract.

138
Decker D, Harper R, Rehfeldt T. EVALUATION OF THE MICROTOX SYSTEM AS A PREDICTOR OF OCULAR IRRITANCY OF TOILETRY PRODUCTS. In Vitro Toxicol 1994;7(2):83-88.

The Microtox in vitro assay system was used to evaluate 58

toiletry and cosmetic products including shampoos, hair conditioners, hairsprays, and skin care products. The Microtox assay assesses light emission changes from exposure of test samples to luminescent bacteria (Photobacterium phosphoreum). The samples were serially diluted in a 2% sodium chloride solution and the bacteria exposed to these dilutions for 15 min. EC50 Values were generated from dose-response curves and plotted against their respective 24 hr unwashed Draize eye irritation scores. Linear regression (R2) values were found to be 0.30 for shampoos, 0.04 for conditioners, 0.07 for hairsprays, and 0.43 for skin care products. With the use of the protocols and statistical methods outlined in this study, the data indicate that the, Microtox assay is not predictive of Draize eye scores for shampoos, conditioners, and hairsprays and has limited value in the prediction of Draize eye scores of skin care products.

139
Oda RM, Ding S, Tran TT, Matsumoto SS, Anger CB. A SIMPLE AND RAPID IN-VITRO ASSAY FOR OCULAR CYTOTOXICITY BASED UPON THE METABOLISM OF CORNEAL CELLS. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):164.

No abstract.

140
Earl LK, Jones PA, Dixit MB, O'Brien K AF. COMPARISON OF FIVE POTENTIAL METHODS FOR ASSESSING OCULAR IRRITATION IN VITRO. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):145.

No abstract.

141
Paller B, Ziets G, Spence ET, Rachui SR, Duke MA, Robertson WD. PREDICTING OCULAR IRRITATION POTENTIAL OF COSMETICS AND PERSONAL CARE PRODUCTS USING TWO IN VITRO MODELS. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994; 7(2):192.

No abstract.

142
Gautheron P, Giroux J, Cottin M, Audegond L, Morilla A,

Mayordomo-Blanco L, Tortajada A, Haynes G, Vericat JA, et al.
INTERLABORATORY ASSESSMENT OF THE BOVINE CORNEAL OPACITY AND PERMEABILITY (BCOP) ASSAY. Toxicol in Vitro 1994;8(3):381-92.

A multinational interlab. study to investigate the bovine corneal opacity and permeability (BCOP) assay is presented. The aim of this work was to det. the capability and possible limitations of this method to predict ocular irritancy of a large set of chems. The assays were carried out in 12 European labs. with different types of activity. In each of these labs. 52 substances, with a wide range of structure, phys. form and irritant properties, were tested and in vitro scores were compared with those obtained from concurrent rabbit eye (Draize) tests. The technique was easily learned by workers in the participating labs., as shown by the fact that there were consistent responses between treated corneas within an individual lab. Interlab. variability was also very good. It was found that a given lab. had a 96% chance of classifying irritants or non-irritants similarly to the other labs. In addn., it was obsd. that corneas preserved overnight responded similarly to freshly prepd. tissues, thus allowing flexibility for those labs. where the availability of corneas is limited. Comparisons between in vivo and in vitro data showed that the BCOP data correctly predicted whether a compd. would be irritating or non-irritating for 44 of the 52 compds. (84.6%). Specificity and sensitivity were also greater than 84%, and the same no. of substances were overestimated as were underestimated (four out of 52). All of the false negatives were solids whereas most of false positives were liqs., indicating that some adjustment in the protocol may be required depending on the phys. state of the substance to be tested. All of the substances selected could be evaluated, with no limitation such as color, insoly., low or high pH. Given the no. of products evaluated and the reproducibility within and among the labs. involved, the overall results are quite satisfactory and therefore confirm the usefulness of the assay for screening chems. for ocular irritation.

143
Gettings SD, Dipasquale LC, Bagley DM, Casterton PL, Chudkowski M, Curren RD, Demetrulias JL, Feder PI, Galli CL, Gay R, et al.
THE CTFA EVALUATION OF ALTERNATIVES PROGRAM: AN EVALUATION OF IN VITRO ALTERNATIVES TO THE DRAIZE PRIMARY EYE IRRITATION TEST. (Phase II) OIL/WATER EMULSIONS. Food Chem Toxicol 1994; 32(10):943-76.

The Cosmetic, Toiletry and Fragrance Association (CTFA) Evaluation of Alternatives Program is an evaluation of the relationship between Draize ocular safety test data and comparable data from a selection of in vitro tests. In Phase II, 18 representative oil/water-based personal-care formulations were

subjected to the Draize primary eye safety test and 30 in vitro assay protocols (14 different types of in vitro endpoints were evaluated; the remainder were protocol variations). Correlation of in vitro with in vivo data was evaluated using analysis of sensitivity/specificity and statistical analysis of the relationship between maximum average Draize score (MAS) and in vitro endpoint. Regression modelling is the primary approach adopted in the CTFA Program for evaluating in vitro assay performance. The objective of regression analysis is to predict MAS for a given test material (and to place upper and lower prediction interval bounds on the range in which the MAS is anticipated to fall with high probability) conditional on observing an in vitro assay score for that material. The degree of confidence in prediction is quantified in terms of the relative widths of prediction intervals constructed about the fitted regression curves: the narrower the prediction interval, the more predictive of the Draize score is the in vitro test result. 16 assays were shown to have the greatest agreement with the Draize procedure and were therefore selected for regression analysis. Based on the magnitude of the 95% prediction bounds of each of the 16 selected assays over the range of test data, it may be inferred that prediction of MAS values from experimentally determined in vitro scores is more accurate for oil/water-based formulations with lower rather than higher irritancy potential. The assays selected for modelling in Phase II generally exhibited weaker relationships with MAS than those selected in Phase I (evaluated using hydroalcoholic formulations), even though several assays were common to both Phases.

144
Oshima H, Nakamura M. A STUDY ON REFERENCE STANDARD FOR CYTOTOXICITY ASSAY OF BIOMATERIALS. Biomed Mater Eng 1994; 4(4):327-32.

The objective of the present study was to find a standard substance for use as a reference in the cytotoxicity assay of biomaterials, as an alternative to animal experiments in recent years. Eight kinds of rubber were made in a plate shape to keep their surface area at 1 cm2 against 10 ml of extract volume. They were extracted by the following three extraction methods (a) dynamic extraction at 200 rpm gyration on alumina balls at 37 degrees C for 24 h; (b) static extraction at 37 degrees C for 24 h and (c) extraction by heating in an autoclave at 121 degrees C for 60 min. At the end of each period each extract was examined for cell viability based on an evaluation by neutral red uptake. These methods were repeated up to seven times. Two kinds of chemicals were also tested. The extracts obtained were used to treat human gingival fibroblasts that have been cultured with DMEM supplemented with 5% fetal bovine serum into a 96 well tissue culture plate by 1 x 10(5) cells/ml, in an incubator

aerated with 5% CO2, and 95% humidified air at 37 degrees C for 48 h. The extracts of ethylene-propylene, butyl, nitrile rubbers, and two kinds of chemicals yielded strong cytotoxicity in all three kinds of extraction methods, while chloroprene, fluorine-contained, isoprene, India, and silicone rubbers showed little cytotoxicity. The results obtained by the three kinds of extraction methods revealed no differences in the order of cytotoxicity of the materials tested. (ABSTRACT TRUNCATED AT 250 WORDS)

145
Chu IH, Toft P. RECENT PROGRESS IN THE EYE IRRITATION TEST. Toxicol Ind Health 1993;9(6):1017-25. (43 REFS)

The rabbit eye irritation test based on the Draize method is required for the hazard assessment of chemicals and products that may come into contact with the eye. Due to the potential for the suffering of animals and subjectivity of the test, many modifications of the method have been made that involved a reduction in the number of animals and a refinement of techniques. Additionally, there has been significant development of in vitro alternatives. This paper reviews recent advances in the in vivo test and in vitro alternatives, as well as regulatory requirements. While the refinement of in vivo protocols has resulted in a reduction in the number and discomfort on animals, the development of in vitro alternatives could lead to an eventual replacement of animal studies. In view of the inherent simplicity of many in vitro methods, some of which comprise cell cultures, further research into the relevance/mechanism of effects is required. Batteries of in vitro tests, when properly validated, may be considered as replacements for animal testing.

146
Rachui SR, Robertson WD, Duke MA, Heinze J. PREDICTING THE OCULAR IRRITATION POTENTIAL OF SURFACTANTS USING THE IN VITRO SKIN2 MODEL ZK 1200. J Toxicol Cutaneous Ocular Toxicol 1994; 13(3):215-220.

Eight raw materials (seven nonionic surfactants and one anionic surfactant) were evaluated in Skin2 model ZK1200 to determine their in vitro ocular irritation potential. In vivo ocular irritation scores for these surfactants ranged from nonirritating to severely irritating. Cytotoxicity values, obtained via MTT reduction, were used to rank order the test materials as well as classify and group them according to their ocular irritation potential. The human skin model and protocol used in this research appear to be promising in vitro tools for categorizing surfactants and offer a high degree of correlation with published in vivo results.

ORGAN CULTURE

147

De Silva O, Perez MJ, Dossou KG. LOCAL LYMPH NODE ASSAY STUDY ON THE IN VITRO RESPONSE TO SENSITIZING CHEMICALS. World Congress

on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994; 7(2):155.

No abstract.

REPRODCUTIVE TOXICITY

148

Esterhuizen AD, Bosman E, Botes A DE, Groenewald O LA, Giesteira M VK, Labuschagne G PJ, Lindeque HW, Rodriques FA, Janse Van Rensburg JJ, Van Schouwenbrug J AM. A COMPARATIVE STUDY ON THE DIAGNOSTIC SENSITIVITY OF RODENT SPERM AND EMBRYOS IN THE DETECTION OF ENDOTOXIN IN EARLE'S BALANCED SALT SOLUTION. J Assisted Reprod Genetics 1994; 11(1):38-42.

The bioassay used by most IVF units to assess culture media is the mouse embryo test. The limitations of this assay are well known. The objective of this study was therefore to evaluate the in vitro bioassay potential of rodent sperm, in terms of relative sensitivities to endotoxins, and to compare the results with the routine mouse embryo and human sperm tests. Results: The greater sensitivity of rodent sperm than of mouse embryos was evident in this study. A further advantage in using the mouse sperm test was the time (4-6 hr) in which endotoxins could be detected. Conclusion: This rapid sperm test proved to be inexpensive, convenient, and invaluable for detecting potential sources of cytotoxicity.

149

Prien SD, Canez MS, Modesitt PT, San Diego J, Messer RH. CAN CULTURED HUMAN LUTEINIZED-GRANULOSA CELLS BE USED TO DETECT TOXINS TO HUMAN REPRODUCTION? Twenty-seventh Annual Meeting of the Society for the Study of Reproduction, Ann Arbor, Michigan, USA, July 24-27, 1994. Biol Reprod 194;50(Suppl. 1):107.

No abstract.

150

Chou K. AUTOMATED IN-VITRO TEST SYSTEM FOR CHEMICAL TOXICITY IN MAMMALIAN SPERM. U. S. Department of Agriculture/Agricultural Research Service. FEDRIP Database, National Technical Information

Service (NTIS), Springfield, VA; 1994.

The overall objective of the proposed studies are to compare the relative toxicity of three organophosphates in sperm metabolic activity with their relative effects on intracellular calcium concentration and sperm motility. The specific aims are: to determine whether organophosphate insecticides alter sperm motility or velocity. To determine whether organophosphate insecticides alter capacitation related increases in intracellular calcium concentration in the sperm. To examine effects of organophosphate insecticides on energy utilization in capacitating sperm. (Dr. Wang will build the oxygen sensors and provide the software package for the computer aided analysis. APPROACH: Ejaculated boar sperm will be treated with three organophosphate insecticides, and assessed for the parameters listed in the specific aims. These parameters will be related among themselves and with the relative toxicity of the three compounds. -- <PR> PROGRESS: Ethylene Bisdithiocarbamates (EBDCs) are a group of widely-used fungicides for fruits and vegetables. The common metabolite of EBDCs, ethylene thiourea (ETU), has been found in exposed animals and plants, as well as in commercial EBDC formulations. It has been hypothesized that ETU toxicity is related to a highly reactive form of atomic sulfur released during the metabolic formation of ethyleneurea from ETU. The objectives of this study were to examine the ETU effects on fertilizationin vitro and free radical production in ETU treated spermatozoa. Spermwere collected from epididymides of mature B6D2-F1 males. Eggs ere obtained from 4 to 12 week-old females. Sperm lipid-peroxyl radicals were examined by an Varian E-4 ESR spectrometer, using spin Trap alpha-(4pyridyl-1-oxide) N-t-butylnitrone. No inhibitory effects of 1 ppm ETU on fertilization in vitro were observed. At 5, 10, 25 and 50 ppm, fertilization was inhibited to about 80% of that seen in the controls. At 800 ppm, no eggs were fertilized. In the controls, 80 to 90% of the eggs were fertilized. In the control spermatozoa, the production of lipid peroxyl radicals surged at two sampling points: one at the first hr and the other at the fourth hr. Spermatozoa incubated in the 1000 ppm ETU produced more lipid peroxyl radicals than the controls at all sampling points. Instead of two surges, ETU-treated spermatozoa showed only one peak production of peroxyl radicals at 2 hr.

RESPIRATORY TOXICITY
151
Bragadin M, Dell'antone P. A NEW IN VITRO TOXICITY TEST BASED ON THE RESPONSE TO TOXIC SUBSTANCES IN SOLUTIONS OF MITOCHONDRIA FROM BEEF HEART. Arch Environmental Contamination Toxicol 1994; 27(3):410-414.

A simple, rapid and inexpensive in vitro method which may be used

as a biological sensor for the valuation of toxicity of chemical compounds in water is proposed. The method utilizes the response of the respiratory chain of beef heart mitochondria to a wide range of toxic substances. By means of simple voltametric measurements, the method not only gives an indication of toxicity in water but, by using different substrates of the respiratory chain and exploiting some features of the toxic substances, it also allows selective detection of some toxic substance or groups of substances. Since the method reproduces the standard "rainbow trout" test, it may also be proposed as a screening test to precede whole bioorganisms bioassays or as a method to assess the presence of certain toxic substances in water.

SKIN/DERMAL TOXICITY

152
Korting HC, Schindler S, Hartinger A, Kerscher M, Angerpointner T, Maibach HI. MTT-ASSAY AND NEUTRAL RED RELEASE (NRR)-ASSAY: RELATIVE ROLE IN THE PREDICTION OF THE IRRITANCY POTENTIAL OF SURFACTANTS. Life Sciences 1994;55(7):533-540.

A comparative study on the in vitro and in vivo irritancy of anionic, amphoteric and non-ionic surfactants was performed. In vitro ED50 values of the surfactants were determined by two cytotoxicity assays, the dimethylthiazoldiphenyltetrazo-liumbromide (MTT) assay and the neutral red release (NRR) assay on serum-free cultured human foreskin keratinocytes. In vivo human irritancy data were obtained by a 24 hour occlusive patch test in volunteers and the irritant skin response quantified by visual scoring, evaporimetry and colorimetry. A close relationship between the evaluation methods of the patch test was observed (r=0.92 to r=0.96), confirming that the 'bioengineering' methods, such as evaporimetry and colorimetry are suitable for measuring skin irritation. For six surfactants evaluated we found a good correlation (r=0.91) between the ED50 values of the MTT assay and the in vivo irritancy data. The NRR assay yielded less satisfactory correlation coefficients with regard to MTT assay (r=0.42) and in vivo irritancy data (r=0.46). This can be mainly attributed to a misinterpretation of the amphoteric and non-ionic surfactants by the NRR assay. While the NRR assay may better evaluate the anionic surfactants, the MTT assay seems to be more suitable when testing a broader range of chemically diverse surfactants. Limitations of cell culture systems are noted, although the potential usefulness of cultured human skin cells for skin irritancy testing has been clearly demonstrated.

153
Reifenrath WG, Lee B, Wilson D, Spencer T. A COMPARISON OF IN VITRO SKIN-PENETRATION CELLS. J Pharm Sci 1994;83(9):1229-33.

A new low-vol. flow-through diffusion cell (LVFC) was designed to provide accurate detns. of penetrant flux across skin, while minimizing the diln. of penetrant in receptor fluid and eliminating the need for magnetic stirring. The performance of the 0.3-mL LVFC was compared to a magnetically stirred, 4.3-mL high-vol. flow cell (HVFC) and to a magnetically stirred, manually sampled 7.5-mL static cell (SC) with hydrophilic and lipophilic penetrants. The clearance of 14C-labeled benzoic acid from the LVFC and HVFC followed an exponential profile expected for complete mixing when the LVFC and HVFC were run at flow rates of 0.4-0.9 and 4.0-5.2 mL/h, resp. The in vitro dispositions of 14C-labeled benzoic acid and estradiol were detd. in the LVFC and HVFC by applying the compds. to split-thickness pig skin at a 4 mug/cm2 dose. Addnl., the effects of receptor fluid flow rate (1.2 vs 3.5 cell vols./h) and method of skin attachment (O-ring vs compression) were detd. on disposition in the HVFC. The percutaneous penetration of benzoic acid and the residue of estradiol within skin did not differ between the LVFC and HVFC. However, the percutaneous penetration of benzoic acid increased significantly using the O-ring attachment as compared to compression at flow rate of 1.2 cell vols./h. The in vitro permeation of benzoic acid-satd. water and 17beta-estradiol-satd. propylene glycol monolaurate through human epidermis was compared between the LVFC, HVFC, and SC. The LVFC and HVFC had flow rates of 0.9-1.0 mL/h. The static cells were manually sampled; both receptor and donor chamber contents were replaced with fresh liq. at 8-h intervals. The flux profiles calcd. from the output of the LVFC accurately represented those of the SC. The flux profiles calcd. from the output of the HVFC were only comparable following correction for penetrant remaining in the cell. The clearance of benzoic acid, percutaneous penetration of benzoic acid, and penetration of estradiol into skin were similar in theLVFC and HVFC, but the LVFC was superior in requiring no magnetic stirring and approx. one-fifteenth the vol. of receptor fluid. Addnl., skin-flux profiles from the LVFC output ccurately represented those from the other cells, but without the inconvenience of manual sampling (SC) or correction of penetrant (HVFC).

154
Mueller-Decker K, Fuerstenberger G, Marks F. KERATINOCYTE-DERIVED PROINFLAMMATORY KEY MEDIATORS AND CELL VIABILITY AS IN VITRO PARAMETERS OF IRRITANCY: A POSSIBLE ALTERNATIVE TO THE DRAIZE SKIN IRRITATION TEST. Toxicol Applied Pharmacol 1994; 127(1):99-108.

This study is aimed at the development of a cell culture assay which may supplement or replace the animal Draize skin irritancy test. Using human keratinocytes, the measurement of proinflammatory eicosanoid and interleukin-1alpha release and of

the impairment of cell viability have provided a suitable in vitro/in vivo correlation for at least three surfactants. The in vitro study has been extended using structurally unrelated, pharmacologically relevant compounds including ethanol, glycerol,

cyclohexanol, acetone, benzoic acid, phenol, acrylamide, triethanolamine, Tween 80, sodium dodecyl sulfate, benzalkonium chloride, NiSO4, SnCl2, and ZnCl2. Time- and dose-response studies were used to establish half-maximal stimulatory (SC50) and inhibitory (IC50) as well as 10-fold stimulatory (ED10) concentrations for arachidonic acid release, cytotoxicity, and IL-1alpha release, respectively. Based upon these values a similar ranking has been obtained for the mildly acting acetone, ethanol, glycerol, and Tween 80 and for the severely acting A23187, benzalkonium chloride, and sodium dodecyl sulfate. With respect to all other test compounds, substantial variation occurred indicating that all three test parameters provide a more complete characterization of the test compound's potency than a single endpoint. These data are ready to be validated by a controlled clinical study aimed at a qualitative and quantitative evaluation of the symptoms of skin inflammation in volunteers.

155
Edwards SM, Donnelly TA, Sayre RM, Rheins LA. QUANTITATIVE IN VITRO ASSESSMENT OF PHOTOTOXICITY USING A HUMAN SKIN MODEL, SKIN2. Photodermatol Photoimmunol Photomedicine 1994; 10(3):111-117.

The ability to accurately predict the phototoxic potential of personal and skin care products remains a key element in assessing the safety of premarketed products. To find a reliable in vitro alternative test for photoirritancy, the European Commission and the European Cosmetic Association are conducting a 3-year, European validation study. Based on the results of this study, an in vitro photoirritancy method will be selected for incorporation into new international guidelines for photoirritancy testing. As a part of this study, Skin2, a cultured human skin system, was used to evaluate the phototoxic potential of chemicals with known photoirritative properties. The Skin2 ZK1351, a 3-dimensional co-culture system, consists of dermal fibroblasts and a multilayered epidermis comprising differentiated keratinocytes. This product line has previously been used to evaluate the irritative potential of topically applied ingredients and products. In this study, various concentrations of the test chemicals were applied to the epidermal side of the Skin2 tissue for contact times of 1 h or 24 h and then the tissue was exposed to 2.9 J/cm2 of ultraviolet A (UVA) radiation. Treated but nonirradiated tissues were also assayed to predict the cytotoxic potential of the test chemicals, which could mask the phototoxic reaction. After exposure, the

tissue substrates were rinsed free of test chemicals and allowed to recover for 24 h. Following this incubation, the MTT reduction assay was used to assess cytotoxicity. The results of tests using this model skin substrate (Skin2) showed a high degree of correlation with data from human and animal models now used to evaluate the phototoxic potential of chemicals.

156
Whittle E, Basketter DA. IN VITRO SKIN CORROSIVITY TEST USING HUMAN SKIN. Toxicol in Vitro 1994;8(4):861-3.

The in vitro skin corrosivity test (IVSCT) has been shown to be a reliable alternative for detecting substances that would be classified as corrosive in the std. in vivo test. The assay is based on the observation that corrosive substances produce a significant redn. in the transcutaneous elec. resistance (TER) of ex vivo rat skin. The use in the test of human skin to provide a means of direct assessment of potential corrosive substances in humans has been assessed. The testing of 12 substances allowed a TER threshold for in vitro classification as corrosive to be established. In further testing of 15 surfactants (three classified as corrosive) and eight fatty acids substances (four corrosive), the three surfactants but no of the fatty acids were identified as corrosive in vitro. Thus, the fatty acids labeled as corrosive, on the basis of animal data, may be incorrectly classified (i.e. in humans the substances would not lead to a corrosive effect following skin contact). It is envisaged that the IVSCT using human skin will be used together with human patch testing and lead to a redn. in animal testing and a more relevant classification of skin irritation potential to humans.

157
Casterton PL, Potts LF, Klein BD. USE OF IN VITRO METHODS TO RANK SURFACTANTS FOR IRRITATION POTENTIAL IN SUPPORT OF NEW PRODUCT DEVELOPMENT. Toxicol in Vitro 1994;8(4):835-6.

Eleven surfactant raw materials with potential applications in light-duty liq. cleaning products were evaluated in vitro using a human skin analog (ATS SKIN2 Model ZK 1100) for predicting cytotoxicity (MTT redn.) and inflammation [prostaglandin E2 (PGE2) release]. Two of the 11 raw materials, both in the same compd. family, were selected to be individually combined with each of the other nine in a 90:10 (raw:selected raw) mixt. Selection criteria were based on desired performance characteristics and low irritation potential as suggested from the individual surfactant assay data. To det. whether irritation potential was mitigated, MTT and PGE2 scores were again detd. for each of the 18 combinations with the resulting data being compared with the untreated raw material data. A plot of the

data indicated that one of two selected materials may have an

'anti-irritant' effect. For raw materials with intrinsic MTT scores of less than 50 mug/mL and with the original data cor. for possible diln. effects, a statistical comparison between individual raw materials and the two sets of combinations was done using a one-sample anal. Both cytotoxicity (MTT) and inflammation (PGE2) were significantly decreased by the milder of the two selected raw materials.

158
Dooley TP, Gadwood RC, Kilgore K, Thomasco LM. DEVELOPMENT OF AN IN VITRO PRIMARY SCREEN FOR SKIN DEPIGMENTATION AND ANTIMELANOMA AGENTS. Skin Pharmacol 1994;7(4):188-200.

An in vitro cell culture assay was developed to identify inhibitors of melanogenesis and agents which produce cytostatic or cytotoxic effects specifically in melanocytes. A total of 50 compds. related to tyrosine, dihydroxyphenylalanine, and hydroquinone (HQ) were tested in vitro to det. their effects upon a murine melanocyte cell line, Mel-Ab, that produces copious amts. of melanin in culture. The agents that demonstrated an inhibition of growth or pigment prodn. by 50% (IC50) at < 100 mug/mL were considered active. The cytotoxicity of melanocyte- active compds. were also tested in vitro on a control nonmelanocyte cell line (HT 1080), using a simple crystal

violet staining method to quantitate adherent cell no. after treatment. The cell culture assay was validated with known potent melanocyte cytotoxic agents, including HQ and 4-S-cysteaminylphenol (4-S-CAP). Although most cytotoxic chems. were nonspecific in this primary screen (i.e. killing both Mel-Ab and HT-1080 cells), several of the compds. tested exhibited high melanocyte-specific cytotoxicity, similar to HQ and 4-S-CAP. Potentially these compds. may be useful as either antimelanoma or skin depigmentation agents. All of the compds. identified as active in this primary screen were cytotoxic or cytostatic to melanocytes, except for the Me ester of gentisic acid, which uniquely inhibited the de novo synthesis of melanin without cytotoxicity.

159
Rutten AA J, van de Sandt JJ M. IN VITRO DERMAL TOXICOLOGY USING SKIN ORGAN CULTURES. Toxicol in Vitro 1994;8(4):703-5.

In order to reduce animal discomfort and to obtain more quant. endpoints, there is a need for reliable, preferably simple, and inexpensive in vitro alternatives to skin toxicity testing. An in vitro model was developed in which full-thickness skin from

various species can be cultured (rabbit, pig, and human). Subsequent to topically applied test compds., parameters of dermal toxicity were investigated, including cytotoxicity (MTT assay) and the release of inflammatory mediators (HETE's). Moreover, percutaneous absorption and concurrent biotransformation of compds. was studied. MTT conversion was inhibited in a dose-dependent manner following topical application of a wide range of irritants. Repair of initial damage was obsd. to some extent. The eicosanoid 12-HETE, which is thought to play an important role in chemotaxis, is released. Interestingly, the antiinflammatory mediator 15-HETE was released only after a prolonged culture time of 48 h, possibly indicating repair of the induced damage. The metabolic fate of the pesticide propoxur was investigated. Permeation rates were comparable in human and rabbit skin, while pig skin was found to be twice as permeable. Extensive cutaneous metab. was obsd. in all 3 species.

160
Dinh SM, Kachmar DA. SALT CONCENTRATION AND SOLVENT EFFECTS ON THE IN VITRO ELECTRICAL RESISTANCE OF HUMAN SKIN. Polym Mater Sci Eng 1993;70:84-5.

The in vitro elec. properties of human skin were investigated in various solvents and salt concns., using electrochem. methods, in relation to to iontophoretic drug delivery. Increasing ethanol concn. in aq. ethanol could increase the porosity of skin, whereas aq. glycerol and aq. propylene glycol contg. up to 50% solvent did not not produce appreciable changes. When a current was applied, the steady-state elec. resistance of skin was const. at low currents, and decreased with increasing currents. This behavior reflected a transition of transport mechanism, from conduction and diffusion controlled to convection. The presence of electroosmosis supports previous findings that ions are transported through charged and narrow pathways.

161
Lu X, Zhao Z, Zuo H, Chu X. IN VITRO DIFFUSION IN EVALUATION OF VARIOUS FORMULATIONS OF CYCLOSPORIN A. Zhongguo Yaoxue Zazhi 1994;29(8):473-5.

Cyclosporin A ointment was prepd. with Azone and propane-1,2-diol, which acted as penetration accelerants. Its compn. was designed by orthogonal test. The percutaneous release rate on the ointment was detd. in vitro with Holland pig's skin and cadaver skin. The percutaneous release rate of the ointment was detd. in vitro with Holland pig's skin and cadaver skin. The results showed that accumulative release rate of excellent formulation of cyclosporin A was satisfactory.

162
Pershing LK, Parry GE, Bunge A, Krueger GG, Shah VP. ASSESSMENT OF TOPICAL CORTICOSTEROID BIOAVAILABILITY IN VIVO AND IN VITRO. Top Drug Bioavailability, Bioequivalence, Penetration 1993; 351-66. (14 REFS)

No abstract.

163
Behl CR, Char H, Patel SB, Mehta DB, Piemontese D, Malick AW. IN VIVO AND IN VITRO SKIN UPTAKE AND PERMEATION STUDIES. CRITICAL CONSIDERATIONS AND FACTORS WHICH AFFECT THEM. Top Drug Bioavailability, Bioequivalence, Penetration 1993; 225-59. (137 REFS)

No abstract.

164
Gordon VC, Mirhashemi S, Wei R, Harutunian V. A NEW IN VITRO METHOD TO DETERMINE THE CORROSIVITY POTENTIAL OF SURFACTANTS AND SURFACTANT-BASED FORMULATIONS. Comun Jorn Com Esp Deterg 1994;25:11-27.

A new in vitro method, CORROSITEX, has been developed to det. the corrosivity potential of chems. and formulations. This in vitro method assigns corrosive materials into Packing Groups I, II and III as described in United Nations (UN) guidelines or into R34, R35 and noncorrosive groups based on their ability to produce necrosis in three minutes, one hour, four hours or later, resp., on the skin of a rabbit. CORROSITEX consists of two compartments - a Dermal biobarrier and a Chem. Detection System (CDS). The dermal biobarrier has been developed using relevant target macromols. Test chems. and formulations, including solids and liqs., are applied directly to the dermal biobarrier. When the chem. destroys the full thickness of this biobarrier, it is detected by the CDS which produces a simple color change. This color change is visually obsd. and the time required for the color change to occur is recorded in order to assign a packing group. If no color change occurs, the chem. may be noncorrosive. A major study of this in vitro method using 60 corrosive and 15 noncorrosive samples was undertaken. CORROSITEX correctly identified 97.7% of these samples as corrosive or noncorrosive. In addn., this new method correctly assigned 88% of the 60 corrosive materials into the correct packaging group. An initial interlab. study using 38 of these ref. chems. and formulations resulted in a lab-to-lab reproducibility of 93-95%. Studies were performed on 63 raw surfactants including all

surfactant classes and blends. In these studies, 53% of the surfactants were quantified. The concordance of the in vitro results to in vivo results for corrosive and noncorrosive classifications was 93%. Addnl. studies were conducted on more than 61 surfactant-based test materials. In these studies, 100% of these test samples were qualified. In the CORROSITEX System, 54 demonstrated concordance to in vivo corrosive and noncorrosive classification. Only one corrosive was underestd. and six noncorrosives were overestd., of which four were severe irritants. This study demonstrates the utility, reproducibility and relevance of the CORROSITEX method to the prediction of potential dermal corrosion. The use of this in vitro test as a screen or tier approach to the current test method shows substantial promise, particularly for industrial cleaners, surfactants and household products.

165
Shivji GM, Segal L, McKenzie RC, Sauder DN. CUTANEOUS TOXICITY OF SURFACTANTS IN NORMAL HUMAN KERATINOCYTES ASSESSED BY CYTOTOXICITY, ARACHIDONIC ACID RELEASE, AND REGULATION OF INTERLEUKIN-1ALPHA mRNA. Toxicol Methods 1994;4(3):193-203.

It is important to identify in vitro end points that are predictive of in vivo toxicity. Three end points were evaluated: cytotoxicity (crystal violet staining, CVS), the release of [3H]arachidonic acid (AAR), and the regulation of the proinflammatory cytokine interleukin-1alpha (IL-1alpha) message in keratinocytes as a potential in vitro assay for surfactants. Cultured normal human epidermal keratinocytes were treated with various concns. of three surfactants (SDS< Triton X-100, and Tween 20) of different in vivo potencies. The CVS50 1 h (surfactant concn. yielding 50% viability) and AAR50 (surfactant concns. causing 50% release of labeled arachidonic acid), both revealed the same rank order of irritancy, with SDS, Triton X-100, and Tween 20 in decreasing order of irritancy. Regulation of cytokine IL-1alpha mRNA was measured by semiquant. reverse transcription-polymerase chain reaction anal. All three surfactants upregulated the expression of IL-1alpha mRNA when compared with the control at various concns. The CVS (1h) and AAR assays demonstrated utility in rank ordering of chems. from the same class as per in vivo results. At the 24-h time point IL-1alpha message was upregulated and no definite stratification of surfactant-induced IL-1alpha mRNA was seen. These data suggest that the AAR and upregulation of IL-1alpha message are contributing factors in the surfactant-induced irritant/toxic effect.

166
Ramsey JD, Woollen BH, Auton TR, Scott RC. THE PREDICTIVE

ACCURACY OF IN VITRO MEASUREMENTS FOR THE DERMAL ABSORPTION OF A LIPOPHILIC PENETRANT (FLUAZIFOP-BUTYL) THROUGH RAT AND HUMAN SKIN. Fundamental Applied Toxicol 1994;23(2):230-236.

The predictive accuracy of in vitro measurements in estimating dermal absorption has been evaluated in rat and human skin using fluazifop-butyl (FB), a lipophilic model compound, at dosage rates of 2.5, 25, and 250 mug/cm2. In vitro studies used rat and human epidermal membranes mounted in static diffusion cells with radiolabeled FB and receptor fluids of 50% aqueous ethanol (Aq Et), 6% polyethylene glycol 20 oleyl ether in saline (PEG), or tissue culture medium (TCM). In vivo rat studies with radiolabeled FB were carried out to parallel previously published human volunteer studies. For rat skin, in vitro measurements with all types of receptor fluid provide an adequate prediction (generally within a factor of 3) of in vivo absorption. Absorption data for human epidermal membranes with a receptor fluid of Aq Et were adequately predictive of the in vivo absorption. In contrast, membranes with PEG or TCM significantly underestimated the in vivo absorption. The results support the conclusion that in vitro studies are useful to predict in vivo dermal absorption in rat and man, when appropriate receptor fluids are used.

167
Rhoads LS. USE OF HYPOTHERMICALLY STORED KERATINOCYTE CULTURES FOR IN VITRO TOXICOLOGY. J Toxicol Cutaneous Ocular Toxicol 1994;13(3):231-248.

A human epidermal model (HEM) was developed in conjunction with a multiple-endpoint fluorescent assay for use in in vitro toxicology studies. This HEM is comprised of normal human epidermal keratinocytes maintained on an acellular gel overlaid on a microporous membrane. The HEM exhibits many features of a multi-layered epidermis, including stratified cellular layers, desmosomes, a basal lamina, and hemidesmosomes at the gel/basal cell layer interface. The HEM and multiple-endpoint fluorescent assay have been used to examine the toxicity of a sulfur mustard simulant, 2-chloroethyl ethyl sulfide (CEES). A drawback to the use fo this HEM, however, for in vitro toxicology studies is the inability to maintain sample viability during transport at 25~C. Two hypothermic storage solutions were tested for their ability to maintain the viability of the HEM for extended periods at 4~ C. Fluorescence-based viability assays showed that both solutions were useful to maintain HEM for at least 1 week at 4~ C. Electron microscopy was used to examine ultrastructural changes due to hypothermic storage. Stored samples were used in CEES exposure studies to demonstrate the ability to utilize refrigerated HEM for toxicology studies with results comparable to control samples.

168
Rhoads LS, Mershon M, Eichelberger H, Van Buskirk RG, Cook JR. A SYNTHETIC HUMAN EPIDERMAL MODEL CAN DIFFERENTIATE ACUTE FROM LATENT TOXIC EFFECTS OF THE MONOFUNCTIONAL MUSTARD ANALOGUE, 2-CHLOROETHYLETHYL SULFIDE. In Vitro Toxicol 1994;7(2):69-74.

The metabolic indicator alamar blue (AB) was used as a noninvasive, fluorescent probe to analyze the toxic effects of the monofunctional sulfur mustard analogue, 2-chloroethylethyl sulfide (CEES). Data from normal human epidermal keratinocytes (NHEK) exposed to an AB incubation time of 30 min indicated that the dye could accurately estimate cell number as low as 500 cells/well. To determine if AB could reflect toxic episodes to skin cells, confluent NHEK monolayers were exposed to CEES or Triton X-100 for 2 hr. An additional 2 hr incubation with AB demonstrated dose-dependent effects of these toxicants. A synthetic human epidermal model, EpiDerm, was similarly treated with CEES or Triton X-100 and the same samples were assayed with AB at 4, 24, and 48 hr postexposure. EpiDerm had many of the manifestations of in vivo skin including desmosomes, keratin filaments, lamellar bodies, and intercellular lamellae. Although 4 hr were sufficient to render 80 mM CEES-treated EpiDerm samples nonviable, 48 hr were necessary to achieve a similar effect with samples treated with 8.0 or 0.8 mM CEES. Histologic analysis of 48 hr samples revealed complete separation of the EpiDerm from its underlying dermal substitute in specimens treated with 1% Triton X-100 (v/v), 8.0 mM CEES, and 0.8 MM CEES; both 48 hr control and 80 mM CEES samples showed no similar separations. These data, taken together, suggest that the epidermis/substrate separation induced by CEES in vitro is time-dependent and is not necessarily a consequence of CEES toxicity. The implication of this research is that a synthetic human epidermis and fluorescent indicator dyes can be used to compare the mechanisms underlying the acute vs. the latent effects of sulfur mustard (HD) in vitro.

169
Soto RJ, Lake LK, Fell LA, Gordon VC. EVALUATION OF AN IN VITRO SKIN CORROSION ASSAY. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):157.

No abstract.

170
European Centre for Ecotoxicology and Toxicology of Chemicals. PERCUTANEOUS ABSORPTION. ECETOC Monogr 1993;20.

No abstract.

171
Roguet R, Cohen C, Dossou KG, Rougier A. USE OF AN IN VITRO HUMAN RECONSTITUTED EPIDERMIS TO ASSESS THE IRRITATIVE POTENTIAL OF TOPICALLY APPLIED PRODUCTS. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):144.

No abstract.

172
Sladowski DT, Steer SJ, Sladowska BM, Clothier RH, Balls M. DIFFERENCES BETWEEN IMMEDIATE AND DELAYED PHOTOTOXICITY THE CONCEPT OF THE BATTERY OF TWO IN VITRO PHOTOTOXICITY TESTS. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):200.

No abstract.

173
Mershon MM, Rhoads LS, Van Buskirk RG. NEW DYE IMPROVES USE OF HUMAN SKIN CELLS AND FLUORESCENCE SPECTROSCOPY TO MEASURE VESICANT TOXICITY. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):124.

No abstract.

174
Petrali JP, Oglesby SB, Hamilton TA. MORPHOLOGIC ACCOUNTS OF A HUMAN SKIN EQUIVALENT EXPOSED TO MUSTARD GAS. In Vitro Toxicol 1994;7(2):95-98.

TESTSKIN, a commercially available human skin equivalent, was used as a model system to study the temporal anatomical-pathological effects of a single vesicating vapor dose of sulfur mustard (HD). Samples were exposed to 10 mul HD vapor for 8 min and harvested at 1, 3, 6, 12, and 24 hr following exposure. Control samples not exposed to HD were harvested at 0 and 24 hr. Light and electron microscopic analysis revealed that the basal cell of the stratum germinativum was selectively affected beginning at 3-6 hr. These early basal cell changes included, an apparent widening of intercellular spaces; a disabling of desmosomal attachments; rounding of cells, nuclear condensations,

and pyknosis; rearrangement of cytoplasmic tonofilaments to a perinuclear position; and perinuclear blebbing. At 12 and 24 hr, the cytopathology progressed to cytoplasmic vacuolation, swollen endoplasmic reticulum, electron opacities, and necrosis that now involved suprabasal cell layers as well. At the basement membrane zone, cellular debris and cellular fragments accumulated in the area of the lamina lucida that appeared to widen this space, resulting in the formation of a cleft. These results are largely consistent with those reported for animal models and cells in culture. In the course of this morphologic study it was observed that skin structures normally present in vivo were absent or incomplete in the human skin equivalent specimens. These included hemidesmosomes, a basement membrane, anchoring filaments, and anchoring fibrils.

175
Frantz SW, Beskitt JL, Tallant MJ, Ballantyne B. COMPARISON OF HUMAN SKIN PENETRATION IN VITRO WITH IN VIVO AND IN VITRO CUTANEOUS PENETRATION IN RATS WITH THREE COMPOUNDS: ETHYLHEXANEDIOL GLUTARALDEHYDE AND POLYMER JR400. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994; 7(2):152.

No abstract.

176
Donnelly TA, Edwards SM, Sayre RM, Rheins LA. A NOVEL APPROACH FOR THE ASSESSMENT OF PHOTOTOXICITY USING AN IN VITRO HUMAN SKIN MODEL. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):200.

No abstract.

177
Botham PA. THE DEVELOPMENT OF IN VITRO TESTS FOR SKIN IRRITATION. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):200.

No abstract.

178
Moody RP. IN VITRO ALTERNATIVES IN SKIN ABSORPTION STUDIES AN

AUTOMATED HPLC ANALYSIS SYSTEM. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):156.

No abstract.

179
Otulakowski G, Zhou L, Fung-Leung WP, Gendimenico GJ, Samuel SE S, Lau CY. USE OF A HUMAN SKIN-GRAFTED NUDE MOUSE MODEL FOR THE EVALUATION OF TOPICAL RETINOIC ACID TREATMENT. J Invest Dermatol 1994;102(4):515-18.

Cultured human keratinocytes and artificial dermal equiv. maintained in vitro do not perfectly mimic the terminal differentiation patterns and response to drugs obsd. in intact human skin. The authors have made use of human skin grafted onto nude mice to demonstrate that such grafts maintain the pattern of pharmacol. responsiveness to all-trans retinoic acid previously reported in human subjects. The use of a quant. polymerase chain reaction method to measure induction of a retinoic acid responsive gene, cytoplasmic retinoic acid binding protein II, has made it possible to generate objective data suitable for investigations of drug efficacy. This method of using grafted human skin has potential broad applicability for investigation of topical drugs in a no. of therapeutic fields.

180
Tsai J, Flynn GL, Weiner N, Ferry JJ. INFLUENCE OF APPLICATION TIME AND FORMULATION REAPPLICATION ON THE DELIVERY OF MINOXIDIL THROUGH HAIRLESS MOUSE SKIN AS MEASURED IN FRANZ DIFFUSION CELLS. Skin Pharmacol 1994;7(5):270-7.

Relationships are drawn between the extent of topical delivery of test compds. in soln. and the period of residence of their formulation on the skin. The studies were performed using in vitro diffusion cell techniques and a test formulation contg. 2% 3H-minoxidil dissolved in 60% ethanol, 20% water and 20% 14C-propylene glycol. The permeation of propylene glycol was effectively halted upon cleansing the skin surface; the skin had very little reservoir capacity for this substance. However, the rate of delivery of minoxidil was only slowed but not stopped upon cleansing. The suggestion here is that a reservoir of minoxidil is formed in the skin which is capable of sustaining an appreciable input of drug even after the skin's surface is scrupulously cleaned. Assay of epidermal concns. of these species not only confirms the existence of the minoxidil reservoir but also shows that the degree of its tissue concn. is proportional to the time of residence of the

formulation on the skin surface. Reapplication of blank vehicle to the cleansed surface had little to no effect on the permeation of the minoxidil and was similarly without effect on that of propylene glycol. While it comes as no surprise that formulation residence time is an important variable in topical delivery, this study demonstrates the complexities of quant. dependencies of delivery on residence time.

181
Clemessy M, Couarraze G, Bevan B, Puisieux F. PRESERVATION OF SKIN PERMEABILITY DURING IN VITRO IONTOPHORETIC EXPERIMENTS. Int J Pharm 1994;101(Jan 25):219-226. (15 REFS)

To evaluate an in vitro experimental iontophoresis system designed to investigate transport of ionic compounds across hairless mouse skin, Ag/AgCl electrodes and a simple permeation cell design were used to measure morphine hydrochloride flux and to test whether its flux would remain steady with time and if it could be modulated by current intensity. An increase in applied current produced an increase in observed efficiency of iontophoresis. Electrode localization and receptor compartment geometry had an effect on morphine hydrochloride fluxes; however, the use of this permeation cell design did not lead to stabilization of iontophoresis efficiency. Lower morphine hydrochloride fluxes were observed with pulsed current as compared to constant current; however, an increase in applied current produced a linear increase in flux, leading to stability of iontophoresis efficiency. It was concluded that the use of Ag/AgCl electrodes yield stable morphine hydrochloride fluxes, which are maintained for at least 5 h at the current intensity usually required during iontophoresis experiments.

182
Strussmann A, Weissen HJ, Wirtz A. WATER VAPOR PERMEABILITY OF SKIN CARE PRODUCTS IN RELATION TO MOLECULAR AND ENVIRONMENTAL INFLUENCES. Int J Cosmet Sci 1993;15(6):227-233. (7 REFS)

An in vitro method for the characterization of water retention behavior of emollients is described using a polytef (polytetrafluoroethylene) membrane to imitate human skin with the degree of water vapor evaporation determined after impregnation with different emollients. Results obtained from different studies under changing conditions indicate that this is a precise and efficient method that is easy to perform with very simple equipment. It allows the generation of data for the characterization of emollients as an aid for skin care product formulation. Additionally, basic studies on emollient water retention due to exogenous influences are possible.

183
Pearse AD, Edwards C. HUMAN STRATUM CORNEUM AS A SUBSTRATE FOR IN VITRO SUNSCREEN TESTING. Int J Cosmet Sci 1993;15(6):234-244. (16 REFS)

The construction of a dedicated, inexpensive, and portable instrument designed to evaluate sunscreens throughout the UVB and UVA range using both Transpore and an alternative substrate of readily obtainable human stratum corneum as substrates on which to spread the test products is described. The transmission of ultraviolet radiation (UVR) through the substrate of stratum corneum was greater than through Transpore tape. Both substrates demonstrated a good correlation in vivo or expected sun protection factor (SPF) results. However, in 10 of 11 sunscreens tested, the comparison of the 2 substrates demonstrated that the predicted SPF using Transpore tape was consistently higher than that using human stratum corneum. It is postulated that the use of human stratum corneum in in vitro SPF testing systems more closely resembles that of human skin in vivo than does Transpore tape with regard to spreading and absorption of the potential sunscreen product.

184
Garrigue J-L, Nicolas J-F, Fraginals R, Benezra C, Bour H, Schmitt D. OPTIMIZATION OF THE MOUSE EAR SWELLING TEST FOR IN VIVO AND IN VITRO STUDIES OF WEAK CONTACT SENSITIZERS. Contact Dermatitis 1994;30(4):231-237. (28 REFS)

The mouse ear swelling test (MEST) was optimized for use in the in-vivo and in-vitro evaluation of weak contact sensitizers. Female Balb/c-mice were used. Two types of MEST, the classical one with a single application of hapten for the induction of the contact sensitivity (CS) reaction, and a MEST variant involving three cutaneous applications of hapten were used. Topical cutaneous applications on the back versus the abdomen were compared. Haptens studied were 1-chloro-2,4-dinitrobenzene (97007) (DNCB), para-phenylenediamine (106503) (pPD), and isoeugenol (97541) (IE) in the in-vivo tests. Haptens used in the in-vitro tests were 2,4-dinitrobenzene-sulfonic-acid (89021) (DNBS) and IE. T-cell enriched lymph node cells (LNC) were prepared from sensitized mice, and proliferation assays were conducted. Tritiated thymidine incorporation was measured using liquid scintillation counting, and stimulation indexes were calculated. Results showed that the back was a better induction site than the abdomen for pPD and IE. All haptens were applied on the back for the rest of the experiments, and a triple dose protocol was used. Optimal concentrations for induction and elicitation phases were 0.5%/1%, 3%/1%, and 10%/25% for DNCB, pPD, and IE, respectively. Maximum ear swelling was at 24 hours

(hr) with pPD and IE, and at 48hr with DNCB. The CS response was similar in magnitude for all three haptens at the optimal concentrations (0.1% DNCB, 3% pPD, and 10% IE). The reactions were specific to the hapten, and no cross reactions were found. With LN cells from DNCB sensitized mice, addition of hapten in-vitro increased lymphocyte proliferation, but reactions with pPD and IE were only moderate at best and nonsignificant. The authors conclude that the MEST is useful for both the in-vivo and in-vitro assessment of the sensitizing properties of a wide range of contact sensitizers.

185
Kemppainen BW, Terse P, Madhyastha MS, Lenz SD, Palmer WG, Reifenrath WG. IN VITRO ASSESSMENT OF IN VIVO DAMAGE TO THE BARRIER PROPERTIES OF PIG SKIN CAUSED BY A COMPLEX MIXTURE. J Toxicol Cutaneous Ocular Toxicol 1993;12(3):239-248. (12 REFS)

The effects of liquid-gun-propellant (LP), a highly irritating mixture consisting of 60.8% hydroxylammonium-nitrate (13465082), 12.9% triethanolammonium-nitrate, and 20% water, on the barrier function of pig skin was studied in-vivo and in-vitro. LP at 25 microliters per square centimeter (microl/cm2) were applied to the shaved backs of crossbred weanling Yorkshire-pigs daily for up to 5 days. Selected pigs were killed after 1, 3, or 5 days and specimens from control and treated skin sites were taken. They were mounted on a diffusion cell apparatus to which 4 micrograms per square centimeter (microg/cm2) carbon-14 (C14) labeled benzoic-acid was added. The extent of penetration of benzoic-acid derived C14 activity through the specimens was measured for 24 hours. Some skin specimens were examined for gross and histopathological changes. Skin discs obtained from the backs of untreated weanling Yorkshire-pigs were mounted on diffusion cells and treated with 25microl/cm2 LP and 4microg/cm2 C14 labeled benzoic-acid for 1 day. The extent of penetration of radioactivity through the specimens was determined. In-vivo, LP induced erythema and multiple small pustule formation. The changes were most severe on day three. The lesions were manifested histologically as superficial perivascular lymphoplasmacytic dermatitis, mild dermal papillary edema, and slight dermal eosinophilia. LP caused a significant decrease in the barrier properties of the skin as evidenced by the penetration of C14 tagged benzoic-acid through the specimens. The effects were seen at all time points but were most pronounced in skin specimens from pigs treated with LP for 1 day. In-vitro treatment with LP caused a level of penetration of C14 activity that did not differ significantly from that of pigs treated with LP for 1 day. The authors conclude that this in-vitro method which is based on the transepidermal transport of C14 labeled benzoic-acid in pig skin can be used to evaluate the effects of chemical or physical agents on the barrier function of human

skin.

186
Koschier FJ, Roth RN, Stephens TJ, Spence ET, Duke MA.
IN VITRO SKIN IRRITATION TESTING OF PETROLEUM-BASED COMPOUNDS IN RECONSTITUTED HUMAN SKIN MODELS. J Toxicol Cutaneous Ocular Toxicol 1994;13(1):23-37. (9 REFS)

An in-vitro experimental method of exposing volatile, water insoluble, petroleum based products to commercially available, full thickness, reconstituted skin was developed. The skin models tested included skin2 Model ZK1300 and Living Skin Equivalent. The potential of the test materials to produce cytotoxicity through the inflammatory cascade response in these tissue models was measured. Tissue samples were incubated in polyethylene bags to minimize cross contamination and loss of volatile constituents. The test substances were dosed undiluted in triplicate onto the epidermal side of the tissues. Thiazolyl-blue reduction and lactate-dehydrogenase, prostaglandin-E2, and interleukin-1-alpha (IL-1alpha) release into spent media from treated and untreated tissues were used as the measured endpoints. Draize primary dermal irritation index scores were used to correlate the data. Each of the end points measured approximated the skin irritation potential of the test materials, with the exception of IL-1 alpha in the skin2 Model ZK1300. The authors conclude that the test systems used in these studies have the potential to be used as screening tests in the evaluation of skin irritation.

187
Basketter DA, Whittle E, Chamberlain M. IDENTIFICATION OF IRRITATION AND CORROSION HAZARDS TO SKIN: AN ALTERNATIVE STRATEGY TO ANIMAL TESTING. Food Chem Toxicol 1994;32(6):539-42.

To date the use of in vitro/alternative tests to achieve formal classification in the EEC of the toxic properties of new substances is very limited. An opportunity exists in the area of skin irritation/corrosivity to adopt a strategic approach which will limit the need to use animals. The approach would involve use of human skin in vitro to identify corrosive materials followed by ethically approved human patch testing. In the patch test, the irritation potential of the substance or preparation would be judged against a suitable positive irritant control. In addition to the avoidance of the use of animals, a further benefit would be that use of human skin should lead to a more relevant classification of skin irritation/corrosion hazard for humans.

188
De Lange J, Van Eck P, Bruijnzeel P LB, Elliott GR. THE RATE OF PERCUTANEOUS PERMEATION OF XYLENE, MEASURED USING THE "PERFUSED PIG EAR" MODEL, IS DEPENDENT ON THE EFFECTIVE PROTEIN CONCENTRATION IN THE PERFUSING MEDIUM. Toxicol Applied Pharmacol 1994;127(2):298-305.

In order to study the dermal permeation of compounds through the skin, an in vitro model was developed which utilized pig ears perfused with autologous pig blood (de Lange, J., van Eck, P., Elliott, G. R., de Kort, W. L. A. M., and Wolthuis, O. L. (1992). J. Pharmacol Toxicol Methods 27, 71-77). In the present article we investigated to what extent the rate of permeation of xylene through pig ear skin is dependent on the perfusion medium used. Pig ears were exposed to xylene (10 cm2 area) for a 4-hr period (30~C, relative humidity of 40-60%) and the perfusate was analyzed for xylene using gas chromatography. The rates of permeation of xylene for whole blood, blood depleted of white blood cells, and a buffer containing 4.5% albumin were similar (:300 ng/min/cm2). The rate of penetration was fivefold higher when pig plasma was used and ninefold lower when albumin was excluded from the buffer. Using the buffer, we found that the rate of permeation of xylene was proportional to flow (constant protein concentration) and protein concentration (constant flow). Our data demonstrate that the measured permeation rate for xylene is, to a large degree, dependent on the effective protein concentration (mg/min) passing through the ear. Differences in this parameter could explain the variations in rates of permeation found using the different perfusion media. To avoid problems associated with the choice of receptor fluid for permeation experiments, we suggest that full blood remains the vehicle of choice, although the practical perfusion period is limited to about 6 hr. If longer perfusion periods are required, then it should be possible to reproduce results obtained with whole blood by choosing an appropriate buffer.

189
Steer S, Balls M, Clothier RH, Gordon V. THE DEVELOPMENT AND EVALUATION OF IN VITRO TESTS FOR PHOTOIRRITANCY. Toxicol in Vitro 1994;8(4):719-21.

No abstract.

STRUCTURE/ACTIVITY TOXICITY
190
Maring CJ, Grampovnik DJ, Yeung CM, Klein LL, Li L, Thomas SA, Plattner JJ. C-3'-N-ACYL ANALOGS OF 9(R)-DIHYDROTAXOL: SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS. Bioorg Med Chem Lett 1994; 4(12):1429-32.

C-3'-N-acyl analogs of 9(R)-dihydrotaxol were prepd. from 7-triethylsilyl-9(R)-dihydrobaccatin III and the corresponding (eR,4S)-N-acyl-3-(1-ethoxyethoxy)-4-phenylazetidin-2-ones. The analogs were tested in a microtubule assembly assay, and in an in vitro cytotoxicity assay. The higher activities obsd. were for the alkylcarbamate substitutions.

191
Eder E, Hoffman C, Deininger C, Scheckenbach S. RISK ASSESSMENT FOR MUTAGENIC AND CARCINOGENIC ACTIVITIES OF ALPHA,BETA-UNSATURATED CARBONYL COMPOUNDS BY A SCREENING STRATEGY BASED ON STRUCTURE-ACTIVITY RELATIONSHIPS. Toxicol in Vitro 1994; 8(4):707-10.

A speedy screening strategy based on structure-activity relationships and a battery of prescreening tests for a rapid and reliable assessment of the role of these compds. in mutagenesis and carcinogenesis is presented and discussed. In this screening strategy, time-consuming and expensive animal tests are replaced by in vitro test with bacteria and cell cultures. The results of the mutagenicity and genotoxicity tests, as well as the results of the binding studies of alpha,beta-unsatd. carbonyl compds. with DNA components, and the corresponding structure-activity relationships, are presented.

TERATOGENICITY
192
Flint OP, Maclean MH. TERATOGENICITY OF TEA EXTRACT PHENOLS ASSESSED IN VITRO. Thirty-fourth Annual Meeting of the Teratology Society and the Eighteenth Annual Meeting of the Neurobehavioral Teratology Society, Las Croabas, Puerto Rico, June 24-30, 1994. Teratology 1994;49(5):420.

No abstract.

193
Newall DR, Beedles KE. THE STEM-CELL TEST - A NOVEL IN VITRO ASSAY FOR TERATOGENIC POTENTIAL. Toxicol in Vitro 1994; 8(4):697-701.

The stem-cell test is a novel assay for teratogenic potential which uses a propagated cell line. Mouse embryonic stem cells (ESC) are maintained in an undifferentiated state in the presence of leukemia inhibitory factor (LIF). On removing LIF, the cells differentiate into an embryonic endoderm that is morphol. distinct from ESC. Colonies of ESC are maintained from which cells can be harvested daily, and these cells, when washed free

of LIF, form a population of differentiating cells on which the effects of chems. can be tested. The conditions under which differentiating ESC can be substituted for rat primary embryonic cells in a micromass test protocol have been detd., and the effects of 25 compds. investigated in a blind trial. The stem-cell test predicted the teratogenicity of these compds. with a similar sensitivity and specificity to the micromass test, with the advantage that the test uses a propagated cell line; there is no use of animals.

194
Bernardini G, Vismara C, Boracchi P, Camatini M. LETHALITY, TERATOGENICITY AND GROWTH INHIBITION OF HEPTANOL IN XENOPUS ASSAYED BY A MODIFIED FROG EMBRYO TERATOGENESIS ASSAY-XENOPUS (FETAX) PROCEDURE. Sci Total Environ 1994;151(1):1-8.

The frog embryo teratogenesis assay-Xenopus (FETAX), a powerful test for the presence of developmental toxicants, has been modified mainly by performing an in vitro fertilization and increasing the exposure time to 112 h. The modified assay (modFETAX) that presents several advantages over the original FETAX methodol. has been validated by the use of ZnSO4, a std. teratogen for FETAX. The modFETAX has been applied to evaluate the 1-heptanol effects on mortality, malformation and growth inhibition. The results indicate that heptanol causes a significant growth inhibition of Xenopus tadpoles and that LC50 and TC50 at 120 h are, resp., 1.49 and 0.37 mM; the resulting teratogenic index (TI50) of 4.03 suggests that heptanol is a strong teratogen.

195
Ogawa M, Takashima H, Wada A, Nagao T, Mizutani M. A COMPARISON OF IN VIVO AND IN VITRO RESPONSE OF RAT EMBRYOS TO A TERATOGENIC INSULT OF 5-FU. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):127.

No abstract.

196
Iwase T, Arishima K, Ohyama N, Inazawa K, Iwase Y, Ikeda Y, Shirai M, Yamamoto M, Somiya H, Eguchi Y. IN VITRO STUDY OF TERATOGENIC EFFECTS OF CAFFEINE ON CULTURED RAT EMBRYOS AND EMBRYONIC CELLS. J Veterinary Medical Science 1994;6(3):619-621.

The teratogenic potential of caffeine was examined in vitro by a whole embryo culture system (WECS) and an embryonic cell culture system (micromas teratogen assay: MTA) in the rat. In the

WECS, hyperemia of the tail, and a reduction of the placental size was induced by caffeine at concentrations higher than 50 mug/ml; hypoplasia of the forelimb bud was induced at concentrations higher than 100 mug/ml; hematoma in the yolk sac and dysmorphogenesis of the fore- and hind-limb buds, prosencephalon and tail were induced by 200 mug/ml caffeine. In the MTA, even with 200 mug/ml caffeine, the toxicological parameters obtained by proliferation and differentiation assays of the midbrain and limb bud cells were almost the same as in the control. In conclusion, caffeine induced various morphological anomalies, but did not affect proliferation or differentiation of cells in these experimental systems.

TISSUE CULTURE

197

Ruegg CE, Silber PM. USE OF HUMAN LIVER SLICES FOR METABOLISM AND TOXICITY STUDIES. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):178.

No abstract.

198

Baiocco A, Boujon CE, Bestetti GE, Rossi GL. AN "IN VITRO" MODEL FOR COMBINED FUNCTIONAL AND MORPHOLOGICAL INVESTIGATIONS ON THE RAT PITUITARY GLAND. Schweiz Arch Tierheilkd 1994;136(6-7):242-7.

We have developed an alternative model for combined "in vitro" functional and morphological studies on the rat pituitary gland. To test its use for biochemical investigations we studied some aspects of the secretion in pituitary thyrotrophs under basal conditions and after stimulation with TRH. After stabilisation of basal TSH release, the stimulation with TRH significantly increased the TSH secretion. To test the usefulness of our model for morphological investigations the tissue was processed for conventional and immunocytochemical light and electron microscopy at the end of the incubation. Different types of pituitary cell resulted to be well preserved. Our model allows combined biochemical and morphological studies on the pituitary gland and thus reduces the number of animal groups, eliminates the extrapolation of results from different groups and replaces the "in vivo" by an "in vitro" experiment.

199

Fentem JH. THE USE OF HUMAN TISSUES IN IN VITRO TOXICOLOGY. Stirling Scotland UK, 28-29 APRIL 1993, Summary of General Discussions. Human Experimental Toxicol 1994;13(6):445-449.

No abstract.

TOXICITY (General)

200

Calleja MC, Persoone G, Geladi P. HUMAN ACUTE TOXICITY PREDICTION OF THE FIRST 50 MEIC CHEMICALS BY A BATTERY OF ECOTOXICOLOGICAL TESTS AND PHYSICOCHEMICAL PROPERTIES. Food Chemical Toxicol 1994;32(2):173-187. (81 REFS)

In an effort to predict human acute toxicity without the use of in-vivo animal testing, the acute toxic effects of the 50 priority chemicals of the Multicentre Evaluation of In-Vitro Cytotoxicity (MEIC) program were evaluated using three cyst based tests, the Daphnia-magna test, and the bacterial luminescence inhibition test along with an evaluation of physical properties such as the n-octanol/water partition coefficients, molecular weights, melting points, boiling points, and densities. Statistical analysis of experimental data demonstrated that in-vitro tests and rodent tests were better predictors of human toxicity expressed as human acute oral lethal dose (HLD) compared with physicochemical parameters. Batteries resulting from partial least squares were more predictive than in-vivo rodent assays or individual tests. The prediction of HLD as well as human lethal concentrations appeared to be dependent on the test species used. The authors conclude that the human lethal concentration of the 50 MEIC chemicals was best predicted using the molecular weight and the D-magna test. A battery of ecotoxicological tests and physicochemical properties could predict the human lethal doses for the majority of the 50 MEIC compounds.

201

Green MR. PUBLIC SOURCES OF IN VITRO TOXICITY TESTING DATA IN NORTH AMERICA. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):196.

No abstract.

VALIDATION/STANDARDIZATION TESTS

202

Blaauboer BJ, Balls M. VALIDATION PROGRAMMES IN EUROPE. World Congress on Alternatives and Animal Use in the LifeSciences, Baltimore, Maryland, USA, November 14-19, 1993.

In Vitro Toxicol 1994;7(2):189.

No abstract.

203
Ohno Y, Kaneko T, Kobayashi T, Inoue T, Kuroiwa Y, Yoshida T, Momma J, Hayashi M, Akiyama J, et al. FIRST-PHASE VALIDATION OF THE IN VITRO EYE IRRITATION TESTS FOR COSMETIC INGREDIENTS. In Vitro Toxicol 1994;7(2):89-94.

Twelve alternative methods to Draize eye irritation tests were preliminarily validated using 9 coded surfactants and physiol. saline soln. by 20 labs. including a national institute, universities, kit suppliers, and cosmetics companies. The results indicated that intra- and interlab. variances were small as a whole except for the case of hemolysis by Tween 20; rank order of the cytotoxicity potentials of Triton X-100 among samples by cultured cell methods with serum differed greatly from those without serum; correlation coeff. between in vitro methods and in vivo Draize scores is higher than 0.8 in HET-CAM-trypan blue staining method, and cultured cell methods with serum and rank order correlation coeffs. were higher than 0.9; correlation among cultured cell methods using serum was also high (r = 0.92-0.99).

204
Selden JR, Dolbeare F, Clair JH, Miller JE, McGettigan K, DiJohn JA, Dysart GR, DeLuca JG. VALIDATION OF A FLOW CYTOMETRIC IN VITRO DNA REPAIR (UDS) ASSAY IN RAT HEPATOCYTES. Mutat Res 1994; 315(2):147-67.

An in vitro flow cytometric (FCM) DNA repair assay has been developed and validated by comparison to conventional autoradiography (ARG). Both assays measure unscheduled DNA synthesis (UDS). Cultures of hepatocytes from young male Sprague-Dawley rats were exposed to a battery of 26 chemicals plus bromodeoxyuridine (BrdUrd) or 3H-thymidine (3H-dT) for 18-20 h before harvest. Selection of test chemicals was based upon both their genotoxicity classifications and carcinogenicity bioassay results in male rats. DNA repair in chemically treated cultures was detected flow cytometrically by measuring the uptake of BrdUrd in non-replicating (G1, G2, mitotic and 4C) cells. Intracellular levels of incorporated BrdUrd were visualized by immunochemical labeling with fluorescein isothiocyanate (FITC), and total cellular DNA content was simultaneously estimated by counterstaining samples with the nucleic acid intercalator, propidium iodide (PI). Information was obtained from 10(4) cells/sample. Since repairing cells incorporate significantly less BrdUrd per unit of time than replicating cells, low intensity BrdUrd-FITC fluorescent signals from repairing cells are readily discriminated from high intensity signals from replicating cells when displayed on linear univariate histograms.

Further distinction between repairing and replicating cells was achieved by displaying the DNA contents of all cells on linear bivariate histograms. Thus, repairing cells were resolved without subjecting these cultures to agents which suppress replicative synthesis (e.g., hydroxyurea). Results from these concurrent FCM and ARG investigations include the following: (1) conclusions (DNA repair positive or negative) were in agreement, with one exception, cinnamyl anthranilate, for which cytotoxic doses produced a positive FCM response, but lack of intact hepatocytes in parallel ARG preparations prevented analysis; (2) similar sensitivities for most of the positive chemicals were reported; (3) a high correlation (85%) exists between the reported genotoxicity classification and these DNA repair results in the absence of overt cytotoxicity; (4) a poor correlation exists between these DNA repair results and hepatocarcinogenesis (only 4/11 liver carcinogens tested positive) or overall carcinogenesis in the male rat (only 9/21 carcinogens tested positive). This FCM assay provides a rapid, sensitive, safe and reliable means of identifying agents which induce DNA repair in mammalian cells.

205
Holm B, Jensen PB, Sehested M, Hansen HH. IN VIVO INHIBITION OF ETOPOSIDE-MEDIATED APOPTOSIS, TOXICITY, AND ANTITUMOR EFFECT BY THE TOPOISOMERASE I-UNCOUPLING ANTHRACYCLINE ACLARUBICIN. Cancer Chemother Pharmacol 1994;34(6):503-8.

A number of clinically important drugs such as the epipodophyllotoxins etoposide (VP-16) and teniposide (VM-26), the anthracycline daunorubicin and doxorubicin (Adriamycin), and the aminoacridine amsacrine exert their cytotoxic action by stabilizing the cleavable complex formed between DNA and the nuclear enzyme topoisomerase II. We have previously demonstrated in several in vitro assays that the anthracycline aclarubicin (aclacinomycin A) inhibits cleavable-complex formation and thus antagonizes the action of drugs such as VP-16 and daunorubicin. The present study was performed to validate these in vitro data in an in vivo model. At nontoxic doses of 6 and 9 mg/kg, aclarubicin yielded a marked increase in the survival of non-tumor-bearing mice given high doses of VP-16 (80-90 mg/kg) in six separate experiments. In therapy experiments on mice inoculated with Ehrlich ascites tumor cells, aclarubicin given at 6 mg/kg roughly halved the increase in median life span induced by VP-16 at doses ranging from 22 to 33 mg/kg. An attempt to determine a more favorable combination of VP-16 and aclarubicin by increasing VP-16 doses failed, as the two drugs were always less effective than VP-16 alone. The way in which VP-16-induced DNA strand breaks lead to cell death remains unknown. However, VP-16 has been reported to cause apoptosis (programmed cell death) in several cell lines. To ascertain whether the protection

given by aclarubicin could have a disruptive effect on the apoptotic process, we used the small intestine as an in vivo model. Whereas VP-16-induced apoptosis in crypt stem cells was detectable at a dose as low as 1.25 mg/kg, aclarubicin given at up to 20 mg/kg did not cause apoptosis. Indeed, aclarubicin caused a statistically significant reduction in the number of cells rendered apoptotic by VP-16. The present study thus confirms the previous in vitro experiments and indicates the value of including an in vivo model in a preclinical evaluation of drug combinations.

206
Stevens-Burns DY, Donnelly TA, Rheins LA. INTRALABORATORY VALIDATION OF A HUMAN DERMAL MODEL FOR IN VITRO IRRITANCY ASSESSMENT. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):190.

No abstract.

207
Walum E, Clemedson C, Ekwall B. PRINCIPLES FOR THE VALIDATION OF IN VITRO TOXICOLOGY TEST METHODS. Toxicol in Vitro 1994; 8(4):807-12. (32 REFS)

A review on key aspects of validation of in vitro toxicity test methods as they have been presented by different authors during the last decade. The Multicenter Evaluation of In Vitro Cytotoxicity study is presented as a practical case in which several of these aspects have been considered.

XYZ/MISCELLANEOUS
208
Ertel SI, Ratner BD, Kaul A, Schway MB, Horbett TA. IN VITRO STUDY OF THE INTRINSIC TOXICITY OF SYNTHETIC SURFACES TO CELLS. J Biomed Mater Res 1994;28(6):667-75.

A trypan blue inclusion assay was used to measure cell death on poly(dimethyl siloxane) (PDMS), polyethylene (PE), poly(methyl methacrylate) (PMMA), polyurethanes, glass, and glow-discharge-reated polystyrene or poly(ethylene terephthalate). Cell lines used were bovine aortic endothelial, 3T3, mouse peritoneal macrophage, and BHK cells. In the absence of proteins in the media, PDMS, PE, PMMA, and some polyurethanes were consistently found to induce cell death. This toxic effect disappeared if the cells were seeded in serum-containing medium or if concentrated solutions of proteins (albumin, IgG, or fibronectin) were preadsorbed on the materials. The substrate toxicity appeared to

be due to the physical properties of the substrate and not to the release of toxic leachables.

209
Ansari GAS, Gan JC. PLASMA PROTEINS: MARKERS OF CHEMICAL EXPOSURE. Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, University of Texas, Galveston, Texas, Grant No. R01-OH-02149, 7 pages, 1994.

The use of plasma proteins as markers of chemical exposure was studied. The specific areas of investigation were the covalent binding of acrolein (107028) to albumin, the rapid quantitation of acrolein and crotonaldehyde (4170303) modified albumin, the susceptibility to degradation of covalently modified albumin, the development of an enzyme linked immunosorbent assay (ELISA) for styrene (100425), and the preparation and detection of styrene-oxide (96093) specific antibodies. The covalent binding of acrolein to human serum albumin showed four new ninhydrin positive peaks. Study of the effects of acrolein on the ultraviolet absorption and the biological function of albumin to bind fatty acid or bromocresol-green, showed that absorption was increased by approximately 80% at 280 nanometers. The authors suggest that more tyrosine and/or tryptophan residues were exposed as a result of the unfolding of the protein through covalent modification of the lysyl and histidyl residues. The preparation of a styrene-oxide albumin conjugate, the generation of polyclonal antibodies in rabbits, and an ELISA procedure for the detection of antibodies were also discussed.

210
Leanderson P, Wennerberg K, Tagesson C. DNA MICROFILTRATION ASSAY: A SIMPLE TECHNIQUE FOR DETECTING DNA DAMAGE IN MAMMALIAN CELLS. Carcinogenesis 1994;15(1):137-139. (11 REFS)

A new, simple technique for detecting DNA single strand breakage in mammalian cells was developed. The technique, known as the microfiltration assay, was based on filtration of alkali lysed cells through microfilters. It was developed using human bronchiolar 14Br cells. The cells were labeled with carbon-14 tagged thymidine, trypsinized, washed with phosphate buffered saline (PBS), centrifuged, and diluted to a concentration of 2.5x10(6) cells per milliliter (ml). The cells were then exposed to the test agent, after which they were transferred to 0.8 micrometer cellulose-acetate filters mounted in an MF-1 Bioanalytical Systems microfilter device. The cells were washed, lysed and centrifuged to separate damaged DNA from undamaged DNA. The filtrates were then transferred to scintillation vials and mixed with scintillation liquid and counted in a beta counter. The radioactivity in each filtrate was compared with the

radioactivity in an untreated cell suspension that had been transferred directly into a scintillation vial. The extent of DNA single strand breakage was determined from the amount of radioactivity remaining on the microfilter. The amount of single strand breakage measured in the microfiltration assay compared favorably with the amount of DNA damage measured in the conventional DNA precipitation assay. The authors conclude that the microfiltration assay is easy to perform and does not require expensive equipment. It may serve as a complementary, or alternative, technique for assessing DNA damage.

211
Walum E, Clemedson C, Ekwall B. THE MEIC PROGRAM AND ITS IMPLICATIONS FOR THE PREDICTION OF ACUTE HUMAN SYSTEMIC TOXICITY. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):197.

No abstract.

212
Wang X, Sasaki T, Saijo K, Ohno T. CORRELATION OF IN VITRO TOXICITY OF MEIC CHEMICALS DETERMINED BY LACTATE DEHYDROGENASE RELEASE ASSAY WITH ANIMAL AND HUMAN TOXICITY. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994; 7(2):176.

No abstact.

213
Fielder RJ. PROGRESS IN ACCEPTANCE OF IN VITRO STUDIES BY REGULATORY AUTHORITIES. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):194.

No abstract.

214
Gordon VC, Mirshairh A, Shemi S, Wei R, Roth B, Mann M. A NEW IN VITRO METHOD TO PREDICT CORROSIVITY POTENTIAL OF TEST MATERIALS AND TO CLASSIFY CORROSIVES INTO PACKING GROUPS I, II, AND III. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):127.

No abstract.

215
Gordon VC, Mirhashemi S, Wei R, Roth B, Mann M. A NEW IN VITRO METHOD TO PREDICT CORROSIVITY POTENTIAL OF TEST MATERIALS AND TO CLASSIFY CORROSIVES INTO PACKING GROUPS I II AND III. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):127.

No abstract.

216
Keese CR, Giaever I. AN ELECTRICAL METHOD FOR IN VITRO TOXICOLOGY. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):126.

No abstract.

217
Ungar K. THE STATUS OF IN VITRO TOXICITY TESTING DATA BASES IN EUROPE. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):196.

No abstract.

218
Loprieno N, Migliore L, Sbrana I, Barale R, Loprieno G. IN VITRO MAMMALIAN MICRONUCLEUS ANEUPLOIDOGENIC TEST. World Congress on Alternatives and Animal Use in the Life Sciences, Baltimore, Maryland, USA, November 14-19, 1993. In Vitro Toxicol 1994;7(2):124.

No abstract.

219
Campbell SG. A NEW MODEL FOR THE STUDY OF BOVINE STAPHYLOCOCCAL MASTITIS IN VITRO. U. S. Department of Agriculture/Cooperative State Res Ser. FEDRIP Database, National Technical Information Service (NTIS), Springfield, VA; 1994.

The objective of this proposal is to investigate the pathogenesis of bovine mastitis by developing an in vitro model to examine the interactions of Staphylococcal aureus and mammary gland

epithelial cells in culture. APPROACH: The investigator would establish an epithelial cell culture system supported by a bovine collagen matrix. This system would then be used to investigate the adhesion of bacterial pathogens to cultured epithelial cells. Radiolabeled S. aureus would be used to investigate the kinetics of binding. Following validation of the model, the role of staphylococcus toxins would be evaluated by comparing adhesion of toxin deficient mutants with the adhesion of wild type S. aureus, and ultimately the effects of purified alpha and beta toxins on the adherence of toxin negative and positive mutants. The experiments are designed to test the hypothesis that the pathogenesis of S. aureus induced mastitis involves adhesion of the bacterium to mammary gland epitehlial cells. The efforts of adhesion on the epithelium would be determined by studying the kinetics of trypan blue exclusion from cultured epithelial cells and by morphologic changes studied at the light and electron microscopic levels. -- <PR> PROGRESS: The effects of Staphylococcal aureus alpha-toxin on bovine mammary gland epithelial cells were studied in vitro. Bovine mammary gland epithelial cells treated with alpha-toxin incurred extracellular plaque-like lesions and intracellular actin disruption. The extracellular plaques were similar to those observed in cows with mastitis. The alpha-toxin appears to act extracellularly by permeabilizing the plasma membrane. When this occurs, calcium appears to play a role in the disruption of the microfilaments.

220
Blaauboer BJ, Balls M, Bianchi, Bolcsfoldi G, Guillouzo A, Moore GA, Odland L, Reinhardt CA, Spielmann H, Walum E. THE ECITTS INTEGRATED TOXICITY TESTING SCHEME: THE APPLICATION OF IN VITRO TEST SYSTEMS TO THE HAZARD ASSESSMENT OF CHEMICALS. Toxicol in Vitro 1994;8(4):845-6.

A multicenter project has been designed, with the purpose of developing the concept of integrated in vitro testing. The aim is to use non-animal methods to assess the toxicol. properties of chems., and to improve this assessment through the use of knowledge of mechanisms of toxic action. A no. of tests or test batteries were selected within eight areas: basal cytotoxicity, irritancy, developmental toxicol., hepatotoxicity, nephrotoxicity, immunotoxicity, neurotoxicity and biokinetics. For each area, a no. of calibration chems. were selected. In an initial pilot study, the neurotoxic properties of five chems. will be studied in combination with a biokinetic anal., in which blood and brain concns. will be predicted from biokinetic modeling.

221
Tao S. ADVANCES IN THE ALTERNATIVES STUDY OF ANIMAL TOXICITY

TESTING FOR CHEMICALS. Nongyao Kexue Yu Guanli 1994;400(3):38-9.

No abstract.