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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.

BRAIN/CNS

1

Albertini S, Brunner M, Wurgler FE. ANALYSIS OF THE SIX ADDITIONAL CHEMICALS FOR IN VITRO ASSAYS OF THE EUROPEAN ECONOMIC COMMUNITIES' EEC ANEUPLOIDY PROGRAMME USING SACCHAROMYCES CEREVISIAE D61.M AND THE IN VITRO PORCINE BRAIN TUBULIN ASSEMBLY ASSAY. Environ Mol Mutagen 1993; 21(2):180-92.

The authors tested six additional chemicals (acetaldehyde, benomyl, diethylstilboestrol, diethylstilboestrol dipropionate, griseofulvin, and mercaptoethanol) in the in vitro systems of the coordinated programme to study aneuploidy induction sponsored by the Commission of the European Communities using two of the in vitro test systems.

2

Banks WA, Audus KL, Davis TP. PERMEABILTY OF THE BLOOD-BRAIN BARRIER TO PEPTIDES: AN APPROACH TO THE DEVELOPMENT OF THERAPEUTICALLY USEFUL ANALOGS in Peptides (Pergamon) 1992; 13(6):1289-94. (66 REFS)

Peptides have been shown in both in vivo and in vitro systems to cross the blood-brain barrier (BBB) and so affect function on the side contralateral to their origin. Some peptides cross primarily by transmembrane diffusion, a nonsaturable mechanism largely dependent on the lipid solubility of the peptide. Other peptides are transported by saturable systems across the BBB. These transport systems can be in the CNS to blood direction, as in the cases of Tyr-MIF-1 and methionine enkephalin, in the blood to CNS direction, as in the case of peptide T, or bidirectional, as in the case of LHRH. An in vitro model of the BBB has been characterized and used to confirm that peptides can cross the BBB. Results with the model agree with those obtained in vivo and have been used to study the permeability of the BBB to peptides, the effect of peptides on BBB integrity, the cellular pathway peptides and proteins use to cross the BBB and the ability of the BBB to degrade peptides. The in vivo and in vitro methods have been used together to develop halogenated enkephalin analogs that are enzymically resistant, cross the BBB readily to accumulate in areas of the brain rich in opiate receptors, and are powerful analgesics.

3

Tsuchiya T. METHOD OF MICROMASS CULTURE OF MIDBRAIN CELLS AND ITS APPLICATION TO IN VITRO MECHANISTIC STUDY. Teratology 1991;44(6):7B.

The purposes of this report are to examine the relationship between the malformations of the cultured rat embryos and the alterations of Midbrain (MB) cells at the embryonic stage of organogenesis induced by ETU, and to examine the alterations in rat and mouse MB cells induced by ETU and by the serum samples prepared from the animals given ETU. From these experimental results, we clarify that the different sensitivities of the midbrain of these two species may be the main reason that ETU is teratogenic in rats but not in mice. Next, we report that MB are unsuitable for estimating the teratogenic potential of retinoids. The arotinoids did not specifically inhibit cell differentiation but were cytotoxic at high concentrations. Finally, embryolethality of new herbicides was not detected by the micromass teratogen test.

CANCER

4

Dorr RT, Alberts DS. TOPICAL ABSORPTION AND INACTIVATION OF CYTOTOXIC ANTICANCER AGENTS IN VITRO. Cancer 1992;70(4):983-7.

Cytotoxic anticancer agents may pose carcinogenic or teratogenic risks to personnel who prepare or administer drugs to patients with cancer. METHODS: A series of laboratory studies were done to quantify the extent of percutaneous absorption and topical inactivation for various cytotoxic anticancer agents. Topical inactivation of anthracyclines and anthracene DNA intercalating agents was evaluated using Ames bacterial mutagenicity assays and reverse-phase high-performance liquid chromatography measurements. RESULTS: Drug levels passing through human abdominal skin exposed in vitro for 24 hours to 100 micrograms of daunorubicin, doxorubicin, and melphalan were negligible and typically less than the high-performance liquid chromatography sensitivity limit of 1-5 ng/ml (less than or equal to 0.001% possible absorption). Melphalan powder was recoverable from the air near a mortar and pestle used to crush 14 2-mg tablets manually; beyond 12 inches, no airborne drug was recoverable. A standard (4%) concentration of calcium hypochlorite completely inactivated the anthracyclines daunorubicin and doxorubicin but

not mitoxantrone. CONCLUSIONS: In summary, topical cytotoxic drug absorption is negligible (if it occurs at all). However, mechanical manipulations of oral formulations may present a risk of exposure to airborne drug particles. Concentrated calcium hypochlorite is extremely effective in the topical inactivation of certain carcinogenic cytotoxic agents.

5
May RD, Fulton RJ. IN VITRO TUMOR TOXICITY WITH ANTIBODY-TOXIN CONJUGATES. In Vitro Methods Toxicol 1992;9-20. (79 REFS)

This report is about how to prepare and test immunotoxins (ITs) for killing tumor cells in vitro. The selection of toxin and Ab are crucial for constructing a successful IT. The authors have provided detailed methodology for constructing an IT-A using ricin A chain, which is widely used in this field. The purity, Ab binding activity, and cytotoxic efficacy of the IT-As are tested by appropriate in vitro methods. Specificity of killing is demonstrated by a direct IT-A assay, which includes using an isotype-matched negative control IT-A, as well as an Ag-negative cell line. Careful in vitro testing of ITs can predict some of the problems associated with their in vivo applications, such as liver toxicity, and nonspecific binding to nontarget tissues. However, in vitro efficacy may not always be translated into in vivo usefulness.'thus, preclinical in vivo animal models must eventually be developed and tested prior to using ITs clinically.

CARCINOGENICITY

6
Renier A, Yegles M, Buard A, Dong H, Kheuang L, Saint-Etienne L, Laurent P, Jaurand MC. USE OF MESOTHELIAL CELL CULTURES TO ASSESS THE CARCINOGENIC POTENCY OF MINERAL OR MAN MADE FIBERS. Cell Biol Toxicol 1992; 8(3):133-9.

The authors have developed a method to culture rat pleural mesothelial cells (RPMC). They studied the effects of asbestos fibers by the application of in vitro tests formerly developed to determined the genotoxicity and transforming potency of soluble xenobiotics. Moreover, they determined whether RPMC expressed cytochromes P 450 known to metabolize polycyclic aromatic hydrocarbons. This paper reviews

the results obtained so far. It was found that asbestos fibers produced a cell transformation and a genotoxicity characterized by the formation of aneuploid cells, abnormal anaphases, chromosomal aberrations, and DNA repair (UDS). In addition, RPMC expressed different forms of cytochromes P 450. It is suggested that the tumorigenic potency of asbestos fibers may be related to the fiber dimensions, to their surface properties, and to in vivo biopersistence; this term involves the fiber solubility in biological medium and the fiber epuration from the lung by clearance mechanisms.

7
Shepherd JG, Chen JR, Tsao MS, Duguid WP. NEOPLASTIC TRANSFORMATION OF PROPAGABLE CULTURED RAT PANCREATIC DUCT EPITHELIAL CELLS BY AZASERINE AND STREPTOZOTOCIN. Carcinogenesis 1993; 14(5):1027-33.

The role of duct cells in the histogenesis of pancreatic carcinoma was studied using a propagable cultured pancreatic duct epithelial cell line derived from a Fischer-344 rat. Tumorigenic transformation was

induced by treatment with two experimental pancreatic carcinogens, azaserine and streptozotocin, or spontaneously using a 'selective' culture condition. Tumors arising from spontaneously transformed cells were anaplastic carcinomas, while those from streptozotocin-transformed cells were well or moderately differentiated ductal adenocarcinomas. Azaserine-treated cells produced moderately to poorly differentiated adenocarcinomas. Ultrastructural evidence of acinar or endocrine differentiation was absent. The biochemical phenotypes of representative tumor cell lines established from these tumors were studied. The results desribed indicate that azaserine and streptozotocin are potent carcinogens in vitro for cultured rat pancreatic duct epithelial cells, and the phenotype of the tumors is modulated by the method or agent used for their transformation.

8
Pfeifer A MA, Cole KE, Smoot DT, Weston A, Groopman JD, Shields PG, Vignaud J-M, Juillerat M, Lipsky MM, Et al. SIMIAN VIRUS 40 LARGE TUMOR ANTIGEN-IMMORTALIZED NORMAL HUMAN LIVER EPITHELIAL CELLS EXPRESS HEPATOCYTE CHARACTERISTICS AND METABOLIZE CHEMICAL CARCINOGENS. Proc Natl ACAD SCI USA 1993; 90(11):5123-5127.

Normal human liver tissue and cultured human hepatocytes are valuable models to study xenobiotic metabolism and toxicity, but they only have a limited in vitro life-span and are not readily available. This report describes the establishment of replicative cultures of human adult liver epithelial cells in serum-free medium. The longevity of three of these cultures, derived from different donors, was extended by introduction of the simian virus 40 large T antigen gene. Two cell lines, THLE-2 and -3, established with a recombinant simian virus 40 large T antigen virus have undergone> 100 population doublings, are nontumorigenic when injected into athymic nude mice, have near-diploid karyotypes, and do not express alpha-fetoprotein. The cells express cytokeratin 18 and albumin in early passage, whereas higher-passage cells in logarithmic-phase growth also express cytokeratin 19. THLE-2 and -3 cells metabolize benzo(a)pyrene, N-nitrosodimethylamine, and aflatoxin B1 to their ultimate carcinogenic metabolites that adduct DNA, which indicates functional cytochrome P450 pathways. Other enzymes involved in metabolism of chemical carcinogens, such as epoxide hydrolase, NADPH cytochrome P450 reductase, superoxide dismutase, catalase, glutathione S-transferases, and glutathione peroxidase are also retained by THLE cells. Thus, these immortalized human liver cells constitute an in vitro model for pharmacotoxicological studies and for the investigation of etiology and pathogenesis of human hepatocellular carcinoma.

9

Ashby J, Hilton J, Dearman RJ, Callander RD, Kimber I. MECHANISTIC RELATIONSHIP AMONG MUTAGENICITY, SKIN SENSITIZATION, AND SKIN CARCINOGENICITY. Environ Health Perspect 1993; 101(1):62-7.

Twenty organic Salmonella mutagens, seven of which (including benzo[a]pyrene) are established skin carcinogens, and one of which (2-chloroethanol) is a well-defined noncarcinogen to skin, have been evaluated for skin-sensitizing activity using the local lymph node assay. The relative mutagenicity of the agents to Salmonella was also established. Fourteen of the chemicals were positive in the local lymph node assay, including the seven skin carcinogens. The data collected imply that mutagenicity, rather than simply activity in the Salmonella assay, is a primary stimulus for electrophilic sensitization and carcinogenic

initiation in the skin. The authors concluded that genotoxicity data for an agent can provide indications of the agent's potential to induce skin sensitization and that genotoxins which are skin-sensitizing agents have an enhanced potential to initiate skin carcinogenesis. We suggest that common, albeit individually distinct, structure-activity relationships underpin genotoxicity, skin sensitization, and the initiation of skin carcinogenesis. These relationships should simplify the hazard evaluation of chemicals and contribute to a reduction in animal usage. Several predictions of skin carcinogenicity are made based on the data presented.

CELL CULTURE

10

DeGregorio M, Wurz G, Emshoff V, Koester S, Minor P, Wiebe V. A BIOASSAY FOR ANTIESTROGENIC ACTIVITY - POTENTIAL UTILITY IN DRUG DEVELOPMENT AND MONITORING EFFECTIVE IN VIVO DOSING. Breast Cancer Res Treat 1992; 24(1):35-41.

Monitoring effective antiestrogenic activity of the triphenylethylenes in patients with breast cancer is usually determined by the duration of response. The pharmacokinetics of toremifene and tamoxifen have been shown to be highly variable but patient specific. In the present study, we developed a method to accurately assess the antiestrogenic activity of these agents using plasma specimens, cell culture, and cell cycle measurements. Plasma specimens (4-5 mls) obtained from patients receiving toremifene (360 mg/day for 5 days in a phase I trial) or tamoxifen (20 mg/day) were extd. and reconstituted in tissue culture media (4-5 mls), and growth inhibition was determined in estrogen responsive MCF-7 cells. Growth inhibition of plasma specimens containing either toremifene or tamoxifen and their metabolites was also examined. Cell cycle measurements were determined following in vitro exposure with flow cytometric techniques. Results show that a dose-response relationship exists between cell growth inhibition and cell cycle measurements for human plasma with added toremifene or tamoxifen, and also for human plasma specimens containing drug and its metabolites after treatment. This antiestrogenic bioassay can address clinical research problems such as patient-specific pharmacokinetics, dosing compliance, and acquired antiestrogen resistance.

11
Schasteen CS, Donovan MG, Cogburn JN. A NOVEL IN VITRO SCREEN TO DISCOVER AGENTS WHICH INCREASE THE ABSORPTION OF MOLECULES ACROSS THE INTESTINAL EPITHELIUM. J Controlled Release 1992; 21(1-3):49-62.

A major obstacle in the development of many new pharmaceuticals, particularly proteins and peptides, is

their absorption across the small intestine mucosal barrier. We have developed a cell culture screen to discover novel compounds that increase the intestinal absorption of poorly transported molecules. The absorption screen uses Caco-2 cells, a human-derived intestinal epithelial cell line, grown as monolayer cultures on a permeable membrane which separates apical (luminal) and basal (serosal) chambers. The baseline absorption of the Caco-2 cell system was measured by adding marker molecules to the apical chamber and measuring their appearance in the basal chamber. The amount of these marker molecules in the basal chamber after a 1 h (37.degree.) incubation was used as a measure of baseline absorption (<1% of the amt. administered). Known absorption enhancers such as polyoxyethylene-9-lauryl ether (POE-9) increased the transport of both the radiolabeled and fluorescent markers. In the same assay, cytotoxicity of the absorption enhancer was quantitated by measuring the release of cellular lactate dehydrogenase (LDH) into the apical solution. Neither the fluorochromes used for monitoring absorption nor the enhancers themselves interfered with the LDH detection, therefore the effect of agents on absorption and cytotoxicity could be measured simultaneously. This assay has been used as a screen to identify a number of potential absorption enhancers. A modification of the assay was used to assess the reversibility of the increased absorption following removal of the putative absorption enhancer. The cell culture screen should allow the identification of novel nontoxic agents which can be used to facilitate the oral absorption of pharmaceuticals.

12
Hayashi Y, Imai M, Goto Y, Murakami N. PATHOLOGICAL MINERALIZATION IN A SERIALLY PASSAGED CELL LINE FROM RAT PULP. J Oral Pathol Med 1993; 22(4):175-9.
The ultrastructure of crystal formation in association with dental pulp cells isolated from rat incisor was studied in vitro. A clone, RPC-K, was obtained and incubated with Na-beta-glycerophosphate (BGP). Growing

pulp cells showed low alkaline phosphatase (ALP) activity, which began to increase with cell proliferation. Pulp cells formed cell multilayers after day 14 of culture. Mineralized tissues were observed within cell multilayers on day 28 of culture. Vesicular structures were found around degenerate and necrotic cells. Some of these vesicles contained needle-like crystals. Organic structures appeared at the periphery of mineralized tissues with a post-embedding demineralization and staining method. Electron diffraction patterns of the newly formed crystals revealed a pattern consistent with hydroxyapatite (HAP). These findings suggest that the RPC-K cell line might be useful for a model system to investigate pathological mineralization.

13
Ciapetti G, Cenni E, Pratelli L, Pizzoferrato A. IN VITRO EVALUATION OF CELL/BIOMATERIAL INTERACTION BY MTT ASSAY. Biomaterials 1993; 14(5):359-364.

The tetrazolium-based colorimetric assay (MTT test) measures only in vitro living cells and the results are directly related to the number of viable cultured cells. It has been adopted in immunological investigations, cancer research and, recently, biocompatibility evaluation. We used the MTT method with minor modifications to fit it to an in vitro study of biomaterial-cell interactions. The MTT assay was confirmed to be feasible, rapid and reproducible. Moreover, it showed a good correlation with other in vitro proliferation assays, such as the 3H-thymidine uptake assay. By using the MTT method and the ASTM procedure for extracting biomaterials, we quantified the in vitro cell compatibility of different metals and polymers.

14
Guelden M. IN VITRO TOXICITY SCREENING USING CULTURED RAT SKELETAL MUSCLE CELLS: I. SURFACTANTS AND MITOCHONDRIAL POISONS. Toxicol In Vitro 1993; 7(1):25-34.

The suitability of a test system using primary cultures of spontaneously contracting rat skeletal muscle cells for assessing effects of chemicals relevant to their toxicity in vivo was evaluated. The effects of ethanol and dimethyl sulphoxide, selected surfactants (digitonin, sodium dodecyl sulphate and Triton X-100) and selected mitochondrial poisons (antimycin A, 2,4-dinitrophenol and pentachlorophenol) on these cultures were assessed. Three endpoints, (1) spontaneous contractility, (2) gross structural membrane integrity and (3) energy metabolism,

were studied by determining the reduction in the number of contracting muscle cells, leakage of cytosolic creatine kinase and changes in glucose consumption, respectively. Cells were also screened microscopically for morphological signs of intoxication. Exposure times of 1 and 24 hr were used. Contractility and glucose consumption frequently proved to be more sensitive parameters than loss of intracellular creatine kinase indicating cell death. Most often prominent morphological alterations became obvious at concentrations causing minimal cytolethality. Non-cytotoxic functional alterations of the muscle cell membrane could be distinguished from cytotoxic action. The results indicate that the test system is suitable for assessing specific qualitative and quantitative aspects of toxicity at the cellular level.

15

Sun J. STUDY ON THE RECOVERY OF 4 TYPES OF CULTURED CELL AFTER CONTACTING VARIETIES OF DENTAL CASTING ALLOYS. Chung Hua Kou Chiang Hsueh Tsa Chih 1992; 27(4):203-5.

Four types of cultured cell (Gin-1, Chang Liver, HEP-2 and L-929)were used in vitro to determine the cytotoxicity of 12 Chinese-Japanese Dental Casting Alloys from cell recovery ability. A new cytocompatibility detecting method--cell recovery test was established and recommended. This method can be used to observe the state of cell recovery through self growth cycle, diminish the side-effect of dead cell and their products on the living cells, nearly mimic the environment in vivo, and study the inherited toxicology of cells. The application of computer photo-pattern analysis technique can be used to acquire objective and accurate data directly.

16

Van Pelt F N AM, Hassing I G AM, Stelling MA, Seinen W, Blaauboer BJ. INDUCTION OF TERMINAL DIFFERENTIATION IN CULTURED HUMAN KERATINOCYTES BY POLYCHLORINATED AROMATIC HYDROCARBONS AS MEASURED BY CELL SIZE ANALYSIS. Toxicol Appl Pharmacol 1992; 113(2):240-245.

Polychlorinated aromatic hydrocarbons modulate the proliferation and differentiation of human epidermal cells in vivo and in culture. One of the earliest events in the process of terminal differentiation is the increase in cell size. In this report the usefulness of morphometric cell size analysis as a quantifiable marker for chemical-induced differentiation was examined. Concentration-related increases in cell size distribution were induced by 2,3,7,8-

tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) and
2,3,4,7,8-pentachlorodibenzofuran in normal human
keratinocytes and cells from an SV40-transformed
keratinocyte cell line (SVK14) whereas the analog
1,2,3,4-tetrachlorodibenzo-p-dioxin did not affect the
cell size distribution up to a concentration of 100 nM. It
was found that the potency of these compounds relative to
that of 2,3,7,8-TCDD correlated well with the toxicity
equivalency factors observed in other test systems.
Experiments indicate that the keratinocyte cell assay is a
useful method for establishing the relative potency of
various "dioxins" and their mixtures.

17
Collodi P, Kamei Y, Ernst T, Miranda C, Buhler DR,
Barnes DW. CULTURE OF CELLS FROM ZEBRAFISH (BRACHYDANIO
RERIO) EMBRYO AND ADULT TISSUES. Cell Biol Toxicol 1992;
8(1):43-61.

The zebrafish is a popular model for studies of
vertebrate development and toxicology. However, in vitro
approaches with this organism have not been fully exploited
because cell culture systems have been unavailable. We
developed methods for the culture of cells from
blastula-stage diploid and haploid zebrafish embryos, as
well as cells from the caudal and pelvic fin, gill, liver,
and viscera of adult fish. Zebrafish cultures were grown in
a complex basal nutrient medium supplemented with insulin,
trout embryo extract, and low concentrations of trout
and fetal bovine serum; they could not be maintained in
conventional culture medium containing a high concentration
of mammalian serum. Using calcium phosphate-mediated
transfection, a lasmid constructed for use in mammalian
cells was introduced into zebrafish embryo cell cultures and
expressed in a stable manner. Experimental results indicate
that the transfection procedures utilized in mammalian
systems can also be applied to zebrafish cell cultures,
providing a means for in vitro alteration of the genotype
and phenotype of the cells.

18
Miller BE, Miller FR, Machemer T, Heppner GH.
MELPHALAN SENSITIVITY AS A FUNCTION OF PROGRESSIVE
METASTATIC GROWTH IN TWO SUBPOPULATIONS OF A MOUSE MAMMARY
TUMOUR. Br J Cancer 1993;68(1):18-25.

In order to examine in detail the sensitivity to
chemotherapy of tumour cells at various organ sites and at
various stages of metastasis, we have used a series of cell

lines, all selected from sister subpopulations derived from a single mouse mammary tumour, which can be distinguished and quantitated from normal cells and from each other through growth in selective medium. The experimental results indicate that, in heterogeneous tumours, individual sub-populations of tumour cells may respond differently to chemotherapeutic agents at various disease stages. In vitro measures of tumour sensitivity do not predict these changes in in vivo sensitivity. Model systems similar to the one described here may yield information which will eventually be useful in maximizing the efficacy of clinically relevant adjuvant chemotherapy regimens.

CYTOTOXICITY

19

Nostrandt AC, Rowles TK, Ehrich M. CYTOTOXIC EFFECTS OF ORGANOPHOSPHORUS ESTERS AND OTHER NEUROTOXIC CHEMICALS ON CULTURED CELLS. IN VITRO TOXICOLOGY. Journal of Molecular and Cellular Toxicology 1992; 5(3):127-136. (28 REFS)

The in-vitro cytotoxicity of organophosphates and other neurotoxic chemicals in a neuronal cell line was examined. Differentiated SY-5Y cells, a human neuroblastoma cell line, were incubated with 0 to 10(-3) molar (M) mipafox, paraoxon, aldicarb, beta,beta-iminodipropionitrile (IDPN), carbachol, carbaryl, or phenyl-saligenin-phosphate (PSP) for 24 hours. Other cells were incubated with the nonneurotoxicants atropine or verapamil for comparison. Effects on viability were determined using the trypan-blue dye exclusion test. SY-5Y cells were incubated with mipafox, paraoxon, aldicarb, IDPN, or carbachol for up to 14 days. In some experiments, atropine was added to the cultures. The cultures were assayed for acetylcholinesterase (AChE) activity after 10 minutes. The cells were analyzed for intracellular calcium (Ca+2) content after 3, 10, 24, and 48 hours. The cultures were examined for histomorphological changes periodically for up to 14 days. A parallel experiment using chicken brain homogenates was performed and compared with the effects on Sy-5Y AChE activity. Only mipafox, carbachol, carbaryl, and PSP significantly decreased cellular viability, with carbaryl and PSP being the most potent. Carbachol, aldicarb, paraoxon, IDPN, and mipafox inhibited AChE activity to a greater extent in SY-5Y cells than in the chicken brain homogenates, with mipafox, paraoxon, and aldicarb being the most potent. The authors conclude that SY-5Y cells can be used to assess the cytotoxic effects of neurotoxic chemicals, especially esterase inhibitors.

20
Borgs P, Way DL, Witte MH, Case TC, Ramirez G Jr, Witte CL. EVALUATING IN VITRO TOXICITY TO MAMMALIAN ENDOTHELIAL CELLS IN IN VITRO METHODS OF TOXICOLOGY, R. R. Watson, Editor; CRC Press, Boca Raton, Florida, 1992;269-284.
(56 REFS)

The use of endothelial cell cultures to examine the cytotoxic effects of various substances such as chemical pollutants, pharmaceuticals, and environmental gases and toxins is examined in this chapter. The application of parameters and techniques developed for in-vitro cytotoxicity testing using cell types other than endothelial cell cultures is discussed. Methods used to culture endothelial cells are described. Indicators of cytotoxicity and genotoxicity in endothelial cells in-vitro are discussed; such as the identification of morphological changes, biochemical abnormalities, behavioral dysfunctions, and changes in cell proliferation. Specific techniques involved in the evaluation of these parameters are described. The potential usefulness of the system in the evaluation of agents known to be associated with vascular disorders is examined.

21
Giridhar J, Acosta D. EVALUATION OF CYTOTOXICITY POTENTIAL OF SURFACTANTS USING PRIMARY RAT KERATINOCYTE CULTURE AS AN IN VITRO CUTANEOUS MODEL. In Vitro Toxicol 1993; 6(1):33-46.

Primary rat keratinocytes were cultured from skin epidermis of neonatal rat pups by a trypsin digestion method. Two-three day old confluent cultures were treated with five surfactants, anionic, amphoteric and nonionic surfactants, in varying concentrations. Evaluation of cytotoxicity after surfactant treatment was measued by: (i) monitoring leakage of cytosolic enzyme lactate dehydrogenase (LDH) into the medium, (ii) mitochondrial reduction of 3-(4-5-dimethythiazol-2yl-)-2,5 diphenyl tetrazolium bromide (MTT) and (iii) lysosomal uptake of the dye neutral red (2-amino-3-methyl-7-dimethyl-amino-phenazoniumchloride) (NR), at the end of a 1 hr treatment period and after 24 hrs immediately following treatment and removal of surfactants. Additionally, changes in cell morphology were assessed by light microscopy. Dose-response curves were generated using the ALLFIT curve fitting program and EC50 values were calculated. Results show a potential for causing cytotoxicity among the surfactants in the following way: nonionics = anionics > amphoterics. The findings of the

assays MTT and NR were comparable to the LDH leakage results. We conclude that primary rat keratinocytes serve adequately as an in vitro model in the screening of surfactants for skin irritancy potential.

22
Barille FA, Arjun S, Hopkinson D. IN VITRO CYTOTOXICITY TESTING: BIOLOGICAL AND STATISTICAL SIGNIFICANCE. Toxicol In Vitro 1993; 7(2):111-116.

This study was designed to determine the potential of an in vitro model for predicting acute human chemical toxicity. Rat lung epithelial cells (L2) were tested for their ability to incorporate radiolabelled amino acids into newly synthesized proteins, in the absence or presence of increasing doses of the test chemical, during a 24-hr incubation. The MTT assay was also performed as a parallel measure of toxicity, IC10, IC50 and IC75 values (10%, 50%, and 75% inhibitory concentrations, respectively) were extrapolated from dose-response curves after linear regression analysis. The biological significance of the results of testing 30 chemicals shows that the experimental IC50 values were more accurate predictors of human toxicity than equivalent toxic blood concentrations derived from rodent LD50s. Overall, the 24-hr protein synthesis experiments were at least as sensitive as the MTT protocol for detecting cytotoxicity. Individually, the toxicity of eight of 15 chemicals was underestimated with the MTT assay. It is anticipated that the procedure, together with a related battery of tests, may supplement or replace currently used animal protocols for human risk assessments.

23
Husoy T, Syversen T, Jenssen J. COMPARISONS OF FOUR IN VITRO CYTOTOXICITY TESTS: THE MTT ASSAY, NR ASSAY, URIDINE INCORPORATION AND PROTEIN MEASUREMENTS. Toxicol In Vitro 1993;7(2):149-154.

Four different in vitro cytotoxicity tests were compared: the MTT assay, the NR assay, the uridine incorporation assay and the measurement of total cellular protein. The comparison was done using the BHK-21 cell line and nine selected test chemicals (colchicine, amitriptyline, cycloheximide, 2,5-hexandione, mercury chloride, cadmium chloride, copper chloride, 2,4-dinitrophenol and chloroquine disphosphate). The concentration that induced 50% inhibition relative to the controls (IC50) was calculated for each test and chemical. The results from the cytotoxicity tests were

generally in good agreement. However, for some chemicals the IC50 values varied significantly between two assays. The largest variation was found for chloroquine diphosphate, where the IC50 value for the NR assay was approximately eight times lower than the IC50 value for the protein measurement.

24
Grant RL, Yao C, Gabaldon D, Acosta D. EVALUATION OF SURFACTANT CYTOTOXICITY POTENTIAL BY PRIMARY CULTURES OF OCULAR TISSUES: I. CHARACTERIZATION OF RABBIT CORNEAL EPITHELIAL CELLS AND INITIAL INJURY AND DELAYED TOXICITY STUDIES. Toxicology 1992; 76(2):153-176 (56 REFS)

The use of rabbit corneal epithelial cell primary cultures in studies on oculotoxic agents was evaluated. Corneal epithelial cells were isolated from New-Zealand-rabbits and maintained in culture. The activities of lactate-dehydrogenase (LDH), aldolase (ALD) and glucose-6- phosphate-dehydrogenase (G6PDH) were studied in cells treated for 1 hour with various surfactants. Cytotoxicity tests were performed up to 24 hours after removal. Neutral-red (NR) and mitochondrial (MTT) cytotoxicity tests were not as effective in predicting the prolonged toxicity of the more irritating surfactants, particularly benzalbronium cloride (BzCl) and sodium dodecylsulfate (SDS), as the LDH leakage test. Ranking of the cytotoxicity of the surfactants based upon these in-vitro assays was the same as rankings reported using Draize in-vivo testing. Morphological examination of the cells 1 hour after treatment with all agents, with the exception of Tween20, showed pronounced formation of pseudopodia and little vacuole formation. The changes induced by the surfactants were more pronounced 24 hours after exposure. The authors conclude that the in-vitro LDH leakage assay is useful in determining the cytotoxic potential of surfactants on rabbit corneal epithelial primary cell cultures.

25
Li AP, Teepe AG. APPLICATIONS OF CULTURED LUNG CELLS IN TOXICOLOGY in In Vitro Methods of Toxicology, R. R. Watson, Editor; CRC Press, Boca Raton, Florida, 1992;253-268. (71 REFS)

The use of purified lung cell populations for cytotoxicity testing and examination of xenobiotic metabolism was examined in this chapter. The isolation and culture of lung cell populations such as Clara cells,

alveolar macrophages, alveolar type-II cells, and endothelial cells was discussed. Clara cells have been particularly useful in xenobiotic metabolic activity studies due to their high level of cytochrome-P450-monooxygenase activity. Due to their phagocytic activity alveolar macrophages have been useful for the evaluation of the cytotoxicity of inhaled gases, dusts and particles. Several cytotoxicity assays involving alveolar macrophages were described. Pulmonary endothelial cells have been primarily used in studies on the mechanisms involved in injuries resulting from oxidant exposure due to their particular sensitivity to such injuries. Mixed cell populations such as epithelial cell explants from the upper airways and lung parenchymal explants have been useful in the study of carcinogenesis and mutagenesis, respectively.

26
Wendt S L JR, Ziemiicki TL, Spangberg LS. INDIRECT CYTOTOXIC EVALUATION OF DENTAL MATERIALS. Oral Surg Oral Med Oral Pathol 1993; 75(3):353-356.

The standard for cellular biocompatibility in vitro testing is American National Standards Institute/American Dental Association Document No. 41a (1982). The standard allows for the contact testing of solid dental materials for cytotoxicity with HeLa or mouse fibroblasts (L929). The purpose of this study was to evaluate a new method of cytotoxicity testing of biomaterials that allows for an economic standardized indirect cytotoxic screening of the originally dispensed material over a period of time at intervals decided by the investigator. The results show a significant difference of cytotoxicity on the basis of the material between the direct contact method and the indirect or diffusion method described.

27
Li X, Li C, Chen Y. TESTING OF CYTOTOXICITY OF HARRINGTONINE AND HOMOHARRINGTONINE ON CFU-GM AND L-CFU WITH MICROLIQUID CLONOGENIC ASSAY IN VITRO. Zhongguo Yaolixue Tongbao 1992; 8(6)459-62.

Based on semisolid agar culture and limiting dilg. microculture, we have established a new chemosensitivity test system in vitro, microliquid clonogenic assay. Using this assay, the cytotoxicity of harringtonine (H) and homoharringtonine (HH) on CFU-GM (colony-forming units of granulomacrophages) and L-CFU (leukemic colony-forming units) were tested. The result suggested that the

cytotoxicity of HH on CFU-GM and L-CFU was more potent than that of H under 24h and 168h exposure. The cytotoxic selectivity of H and HH on L-CFU was larger than on CFU-GM, but the cytotoxicity of HH was smaller than that of H. H and HH were cell cycle phase specific drugs according to their TDI (Time-Schedule Dependence Index). The possibility of homologous drug cross-resistance between H and HH were investigated.

28

Ohsumi T, Soh Y, Higashi S, Ozumi K, Kuroki K. A STUDY ON APPLICABILITY OF SIX ORGANIC SOLVENTS FOR SUBJECT CHEMICALS TO IN VITRO CYTOTOXICITY ASSAYS. Kyushu Shika Gakkai Zasshi 1993; 47(2):305-10.

Cytotoxicity of 6 organic solvents, DMSO, MeOH, HCONH2, EtOH, DMF, and AcOEt, was examined on HeLa cell culture. The cells were exposed to each of these solvents at 1.0-20.0 muL/mL medium during 24 h cultivation, and IC50 was calculated on the basis of the ratio of normal nuclei in the cultures with the solvents added to that in controls. The IC50 values for DMSO, MeOH, HCONH2, EtOH, DMF, and AcOEt were 14.0, 13.0, 10.0, 8.0, 4.0, and <1.0 muL/mL medium, respectively, indicating that their in vitro cytotoxicity increased in this order. These data shows that DMSO has the least cytotoxicity, followed by MeOH and HCONH2, and that these 3 solvents are applicable to test chemicals in cell culture, if used in low concentrations.

29

Babich H, Borenfreund E. NEUTRAL RED ASSAY FOR TOXICOLOGY IN VITRO In IN VITRO METHODS OF TOXICOLOGY. R. R. Watson, Editor; CRC Press, Boca Raton, Florida, 1992; 237-251. (82 REFS)

The neutral red (NR) cell viability assay for the assessment of in-vitro cytotoxicity was examined in this report. The principle of the NR assay was discussed as well as the protocol for performance of the assay. The relevance of data obtained using the NR in-vitro assay to in-vivo toxicity was examined. Strong correlations were seen between data obtained from in-vivo eye and skin irritation tests, irritancy potential of parenteral formulations, and in-vivo renal toxicity tests and those observed after NR testing of the same substances. The use of the NR assay to compare the sensitivities of normal, transformed, and cancerous cell lines to chemotherapeutic agents was examined. The NR assay has also been used to establish structure activity relationships for several series of related compounds.

Approaches that have been used to adapt the NR assay to
examine metabolism mediated cytotoxicity were described and
the use of the NR assay in determining toxicity/temperature
interactions were discussed. Experiments illustrating the
use of a new application of the NR assay in solar simulated
ultraviolet phototoxicity testing to determine cell
sensitivities to ultraviolet radiation were described.

30
Balls M, Clothier RH. CYTOTOXICITY ASSAYS FOR
INTRINSIC TOXICITY AND IRRITANCY In IN VITRO METHODS OF
TOXICOLOGY. R. R. Watson, Editor; CRC Press, Boca Raton,
Florida, 1992;37-52. (44 REFS)

The development and modification of cytotoxicity tests
was discussed in this chapter. Important factors to be
considered in the development of cytotoxicity tests were
examined including the cell type, the endpoint measurements,
the length of exposure, the commercial availability of
required reagents, and validation techniques. Three tests
were discussed in detail that were developed as part of the
FRAME Research Program, the FRAME Kenacid Blue test, the
FRAME Neural Red Release test, and the FRAME Fluorescein
Leakage test as examples of general cytotoxicity tests. The
relevance of general cytotoxicity testing to the estimation
of the acute systemic and local toxicity of a substance
in-vivo was examined. The use of test batteries was explored
and the principles of test battery selection outlined. The
use of general cytotoxicity tests as alternatives to ocular
and dermal toxicity testing in animals and as methods to
study metabolism mediated toxicity was explored. The use of
in-vitro cytotoxicity tests for the determination of
nonacute effects was discussed.

31
Shrivastava R, Delomenie C, Chevalier A, John G, Ekwall
B, Walum E, Massingham R. COMPARISON OF IN VIVO ACUTE
LETHAL POTENCY AND IN VITRO CYTOTOXICITY OF 48 CHEMICALS.
Cell Biology and Toxicology 1992; 8(2):157-170. (26 REFS)

A study of the relationship between the cytotoxicity of
a battery of 48 toxic compounds and their in-vivo acute
lethal potency was conducted. The study was part of the
Multicenter Evaluation of In Vitro Cytotoxicity (MEIC)
program, which was an international collaborative program to
determine if assays of in-vitro toxicity could replace the
classical median lethal dose (LD50) for assessing acute
in-vivo toxicity. Forty eight compounds on the MEIC list
were evaluated. The authors conclude that accurate

LD50s in rats and mice can be predicted from the in-vitro
cytotoxicity data for at least 75% of the MEIC chemicals.

32
Elia MC, Storer RD, Harmon LS, Kraynak AR, McKelvey TW,
Hertzog PR, Keenan KP, DeLuca JG, Nichols WW. CYTOTOXICITY
AS MEASURED BY TRYPAN BLUE AS A POTENTIALLY CONFOUNDING
VARIABLE IN THE IN VITRO ALKALINE ELUTION/RAT HEPATOCYTE
ASSAY. Mutat Res 1993; 291(3):193-205.

Rat hepatocytes treated in vitro with A2RA, an
angiotensin II receptor antagonist, displayed an increased
level of DNA-strand breaks as determined by alkaline
elution, without an appreciable increase in cytotoxicity as
determined by a trypan blue dye exclusion assay at harvest.
The alkaline elution profile appeared to have two
components: a rapidly eluting component detected in
the first fraction collected (often associated with DNA
from dead or dying cells), followed by a more slowly eluting
component detected in the subsequent fractions. Further
analysis of hepatocytes treated with A2RA by pulsed-field
gel electrophoresis and neutral elution revealed significant
levels of DNA double-strand breaks. Electron microscopy (EM)
showed pronounced damage to mitochondria; although cell
blebbing was seen using both EM and light microscopy, the
plasma and nuclear membranes appeared intact when examined
by EM. Although, in the experience of the authors the trypan
blue dye exclusion accurately reflects cytotoxicity induced
by the majority of test agents, in this rather unusual
instance, trypan blue did not accurately reflect
compound-induced cytotoxicity at harvest since there
was no concurrent loss of membrane integrity. The data
collected indicate to the authors that interpretation of the
elution assay as a test for genotoxicity can still be
confounded by the failure of the trypan blue dye exclusion
assay to reflect cytotoxicity in the unusual instance when
there is no concurrent, immediate loss of membrane
integrity.

33
Perocco P, Colacci A, Grilli S. IN VITRO CYTOTOXIC AND
CELL TRANSFORMING ACTIVITIES EXERTED BY THE PESTICIDES
CYANAZINE, DITHIANON, DIFLUBENZURON, PROCYMIDONE, AND
VINCLOZOLIN ON BALB/C 3T3 CELLS. Environmental and
Molecular Mutagenesis 1993;21(1):81-86. (21 REFS)

The effects of exposure to cyanazine, diflubenzuron,
procymidone, dithianon, and vinclozolin on the in-vitro cell
transforming potential in BALB/c-3T3 cells was investigated.

The cell transformation test was performed in the presence or in the absence of S-9 mix as an exogenous bioactivation system for the chemicals. In the absence of S-9, all the chemicals studied were cytotoxic. In the presence of S-9, only dithianon exerted cytotoxic activity. BALB/c-3T3 cell transformation was induced by all the chemicals tested, to varying degrees, in the absence of S-9 mix. In the presence of S-9 it was not possible to detect the cell transforming ability of cyanazine and diflubenzuron. The authors stress that the positive findings reported could be relevant in assessing hazard and risk associated with the human exposure to the tested chemicals. The authors suggest that the chemicals may also act in the environment as cocarcinogenic or syncarcinogenic and thereby interact with other genotoxic or nongenotoxic chemicals. The authors conclude that BALB/c-3T3 cell transformation can be validated as a suitable model for the analysis of carcinogenic potential of many other compounds.

34
Martin A, Clynes M. COMPARISON OF 5 MICROPLATE COLORIMETRIC ASSAYS FOR IN VITRO CYTOTOXICITY TESTING AND CELL PROLIFERATION ASSAYS. Cytotechnology 1993;11(1):49-58.

The paper describes a critical comparative evaluation of 5 miniaturised colorimetric assays applicable to cytotoxicity testing of anti-tumor drugs (and other toxic agents) in vitro. Each assay shows a different linear range for optical density versus cell number, a different sensitivity to change in cell number and a different minimum detectable cell number; the values of these parameters vary with experimental conditions and with cell line used. All the methods gave good correlation with viable cell number (determined by colony forming efficiency) in toxicity assays after 3 or 4 days of treatment, but they underestimated cell death after 2 days. Toxicity levels for individual chemicals (in a standard 6-day assay) are similar for the different assays irrespective of the mechanism of action of the chemical being tested. Two of the more recently developed assays (APNaOH and SRB) were found to be very sensitive under the conditions examined.

35
Laschinski G, Vogel R, Spielmann H. CYTOTOXICITY TEST USING BLASTOCYST-DERIVED EUPLOID EMBRYONAL STEM CELLS: A NEW APPROACH TO IN VITRO TERATOGENESIS SCREENING. Reprod Toxicol 1991;51:57-64.

To develop a mammalian in vitro system for teratogenicity

testing, cytotoxicity of xenobiotics was evaluated in pluripotent euploid embryonal stem cells (ESC) derived from mouse blastocysts. The dimethyl-thiazol-diphenyl tetrazolium bromide (MTT) assay was the most appropriate test system for cytotoxicity determinations with ESC. Only compounds that do not require metabolic activation were selected for testing from the database for validation of in vitro teratogenesis assays by Smith et al. Results obtained with ESC were compared to corresponding data from broblasts from day-14 mouse embryos to detect differences in sensitivity between undifferentiated and differentiated cells. ESC showed a higher sensitivity to known teratogens than fibroblast cultures, which allows calculation of a sensitivity ratio of "adult" cells (differentiated fibroblasts) to embryonal cells (undifferentiated ESC) in a mammalian system similar to the hydra assay. Although some xenobiotics had to be classified as false negatives, the ESC cytotoxicity assay holds promise as a new in vitro screening assay in teratology.

DERMAL TOXICITY

36

Ponec M. IN VITRO CULTURED HUMAN SKIN CELLS AS ALTERNATIVES TO ANIMALS FOR SKIN IRRITANCY SCREENING. Int J Cosmet Sci 1992;14(6):245-64. (69 REFS)

In the last few years a lot of attention has been paid to the development of the in vitro models which would substitute for animals in cutaneous irritancy studies. These models explore either organ or explant cultures using freshly excised skin or serial cultures of isolated skin cells (epidermal keratinocytes or dermal fibroblasts). The organ or explant models are suitable only for short exposures of skin samples to the compounds tested and the use of it will always be restricted by the limited availability of fresh human skin. The model that uses submerged cultures of keratinocytes or fibroblasts permits the production of a large number of cells, and permits large scale toxicity screening tests with many substances, that can be applied in a broad concentration range. Since the stratum corneum is absent in conventional (submerged) keratinocyte culture systems, this model is mainly suited for testing of water soluable compounds and it is less suitable for poorly soluble compounds and for topical products consisting of complex formulations which are made of active ingredients and their vehicles. This shortcoming can be overcome by using organotypic cultures in which keratinocytes are grown at the air-liquid interface on a suitable dermal substrate. Under these conditions, the culture forms a multilayered epidermis showing an overall

structure which resembles that of native epidermis. For the evaluation of toxicity a number of tests have already been developed: assessment of cell viability, changes in cell morphology, modulation of cell proliferation and differentiation, monitoring of membrane damage, the measurements of the uptake or incorporation of radioactive precursors, establishment of the modulation of cell metabolism, determination of the release of inflammatory mediators, etc. All these in vitro techniques are still in a state of validation as far as their predictive value for in vivo skin irritancy is concerned.

37
Gueniche A, Ponce M. USE OF HUMAN SKIN CELL CULTURES FOR THE ESTIMATION OF POTENTIAL SKIN IRRITANTS. Toxicol In Vitro 1993;7(1):15-24.

We have investigated the use of cell cultures derived from human skin (keratinocytes and fibroblasts) and of cultured SV40 transformed human keratinocytes (SVK14 cells) for in vitro screening for skin toxicity. To assess the cytotoxic effects of a number of surfactants the following cytotoxicity assays were performed: (1) changes in cell morphology and cell number in confluent cultures; (2) inhibition of cell proliferation; (3) modulation of interleukin-6 (IL-6) production. The data obtained in vitro were compared with the results of an in vivo study (Tupker et al. Contact Dermatitis 1989, 20, 108-114), in which visual scoring and measurement of transepidermal water loss were taken as measures of skin irritancy. The results of the morphological and proliferation studies in vitro indicated a similar ranking order in skin irritancy as that found in the vivo study. Furthermore, IL-6 production by cultured skin cells was increased on administration of nearly all of the surfactants tested. For a number of surfactants the increase in IL-6 production occurred at much lower concentration than those necessary to induce cytotoxic effect, suggesting that monitoring the IL-6 production on administration of skin irritating agents could be used as a very sensitive test.

38
Mueller-Decker K, Fuerstenberger G, Marks F. DEVELOPMENT OF AN IN VITRO ALTERNATIVE ASSAY TO THE DRAIZE SKIN IRRITANCY TEST USING HUMAN KERATINOCYTE-DERIVED PROINFLAMMATORY KEY MEDIATORS AND CELL VIABILITY AS TEST PARAMETERS. In Vitro Toxicol 1992;5(4):191-209.

The authors propose to use 3 mechanistically independent in

vitro parameters of irritancy which reflect major characteristics of acute inflammation i.e. the conconcentration of (1) key mediators of the eicosanoid cascade and (2) of interleukin-1alpha released upon challenging cells in vitro with test substances, and (3) the toxic insult on these cells to monitor skin irritancy. The permanent human foreskin-derived keratinocyte cell line HPKII was used for the establishment of the routine test. Upon challenge with exogenous irritant compounds. HPKII are shown to release arachidonic acid and PGE2 and the proinflammatory cytokine interleukin-1.alpha. Measuring proinflammatory mediator release and cytotoxicity in these cells may prove, therefore to be suitable as test system for the prediction of the irritant potential of chemicals.

39
Lawrence JN, Benford DJ. CHEMICALLY-INDUCED UNSCHEDULED DNA SYNTHESIS IN CULTURES OF ADULT HUMAN EPIDERMAL KERATINOCYTES. Mutat Res 1993; 291(2):105-15.

Skin is a major target organ for many experimental carcinogens that exist in our environment and the majority of previous carcinogenicity studies have utilized animal derived models. In view of the fact, that many of these environmental chemicals exhibit species- and tissue-specific metabolism, a human skin tissue derived model would be a distinct advantage. Squamous epithelial carcinoma is a predominant form of skin cancer in man and, in theory, human epidermal keratinocytes present an appropriate target cell to employ as an in vitro system to study epidermal carcinogenesis. This report demonstrates the valuable potential of human keratinocyte cultures as a suitable model for mechanistic studies on factors which may influence DNA damage and, hence, the subsequent development of cancer in human epidermis.

40
Gerberick GF, House RV, Fletcher ER, Ryan CA. EXAMINATION OF THE LOCAL LYMPH NODE ASSAY FOR USE IN CONTACT SENSITIZATION RISK ASSESSMENT. Fund Appl Toxic 1992; 19(3):438-445.
(38 REFS)

A lymph node assay for detecting contact sensitizers was developed. Female CBA/J-mice were treated once daily for 4 consecutive days with 12.5 microliters of the test agent applied to each side of both ears. Eighteen to 24 hours after the last application the mice were injected intravenously with tritium labeled thymidine. The mice were

killed 5 hours later and the bilateral auricular lymph nodes were removed. The extent of lymph node cell proliferation was determined by measuring uptake of tritium activity. Chemicals that caused a greater than 30 fold increase in tritium uptake were considered to be moderate to strong sensitizers. Chemicals that caused a 2 to 30 fold increase in tritium uptake were considered to be weak to mild sensitizers. The assay was tested with 17 chemicals, 14 of which were known to be contact sensitizers. Results indicated that the assay was useful for identifying strong, moderate, and some weak sensitizers. The authors conclude that the lymph node assay is a viable alternative to currently used test methods for assessing the risk of contact sensitization from chemical agents.

41
Laska DA, Poulsen RG, Horn JW, Meador VP, Hoover DM.
AN EVALUATION OF TESTSKIN: AN ALTERNATIVE DERMAL IRRITATION MODEL. In Vitro Toxicol 1992; 5(4):177-89.

Prediction of severe or corrosive chemical-induced dermal reactions was evaluated in an in vitro model. TESTSKIN, a human living skin equivalent consisting of differentiated human keratinocytes grown on a human fibroblast and bovine collagen matrix coated filter support, provided a model system that resembles many aspects of mammalian skin. Cellular viability and mitochondrial function, as well as histological and ultrastructural characterization of tissue morphology, were evaluated following incubation of TESTSKIN with 9 test articles chosen based on their previously reported animal dermal irritation category. Various incubation times, doses, and dose administration techniques were evaluated. Morphological changes were observed to precede measurable differences in cellular viability or mitochondrial function. Viability measurements and mitochondrial function were generally correlative. A single test article dose of 25 mg or 25 muL of each of 9 test articles provided data that best correlated with the established test dermal irritation category.

42
Reece BT, Deeds D, Rozen M. IN VITRO METHOD FOR SCREENING SUNSCREEN FORMULATIONS FOR SUN PROTECTION FACTOR USING A FULL-THICKNESS SKIN MODEL. J Soc Cosmet Chem 1992; 43(N/D):307-312. (18 REFS)

To determine if artificially grown human skin could provide a useful model system for screening sun protection

factor (SPF) formulations, a 3-dimensional artificial skin model was used to determine the SPF of various sunscreens by measuring the effect of timed UV exposure versus skin responses (IL-1alpha release). No IL-1alpha was detected after 2 min of exposure; however, by 4 min almost 8 pg/ml of IL-1alpha was present in the medium. Formulas without sunscreen active ingredients did not prevent the release of IL-1alpha while those products containing sunscreen active ingredients were successful in preventing its release. No decrease in viability was seen in skin protected by sunscreen; however, skin not protected by sunscreen showed a decrease in viability after 11 minutes of exposure. It was concluded that this method may prove useful as a tool for screening the SPF of sunscreens.

43

Botham PA. CLASSIFICATION OF CHEMICALS AS SENSITISERS BASED ON NEW TEST METHODS. Toxicol Lett 1992; (64/65):165-171. (35 REFS)

New methods that have been developed for the identification and classification of chemicals as skin or respiratory sensitizers were reviewed. Current regulations regarding the testing of potentially sensitizing substances were discussed. These regulations required the use of seven tests performed on guinea-pigs. A brief discussion and review of the immunological mechanisms involved in the induction of allergic reactions by chemicals was presented. Techniques that have been used for the evaluation of respiratory sensitization potentials have included: intradermal injection and assessment of antibody responses, and a four tier approach involving the analysis of the structure/activity relationships of a chemical, its potential to haptenate proteins in-vitro, its immunogenicity using a guinea-pig injection model, and its allergenicity using a guinea-pig inhalation model. New test guidelines for skin sensitization testing published by the OECD in 1992 were discussed.

44

Perkins MA, Osborne R. DEVELOPMENT OF AN IN-VITRO METHOD FOR SKIN CORROSION TESTING. Annual Meeting of the Society for Investigative Dermatology, Washington, D.C., USA, April 28-May 1, 1993. J Invest Dermatol 1993; 100(4):535.

No abstract.

45
Fedorov SM, Selisskii GD, Somov BA. PREVENTION OF OCCUPATIONAL ALLERGIC DERMATOSIS IN CONSTRUCTION INDUSTRY. Vestn Dermatol Venerol 1992; 0(10):24-27.

Occupational dermatoses rank first in the occupational morbidity structure among construction industry workers, their share in this structure being 35%. Employment of formaldehyde-containing plasticizers in cement making has made the problem of occupational cement-induced dermatoses still more pressing. In vitro tests are used to diagnose occupational diseases of the skin in construction workers. The most informative are the hematologic tests and cytochemical investigations of leukocytic acid phosphatase and myeloperoxidase levels. The method of six-valence chromium inactivation in cement via introduction of iron monoxide sulfate, a metallurgic industry product, was effectively used for the first time in this country.

46
Lockhart BP, Bailey CJ. ELISA ESTIMATION OF THE BINDING OF EPIDERMOLYTIC TOXIN TO NEONATAL MOUSE SKIN. Toxicon 1993;31(5):569-76.

A sandwich ELISA, with antisera from rat and rabbit, was used to detect epidermolytic toxin (ET) to a limit of about 0.01 ng at 0.1 ng/mL. The binding of ET to the epidermis of skin disks was measured in vitro. The ability of the assay to discriminate between the two forms of the toxin was used to demonstrate that there was a saturable component of toxin binding to the epidermis. The rate of uptake, the effect of the inhibitor EGTA and comparative expteriments with the inactive nitrated toxin confirmed that the observed binding is associated with toxigenesis. From measurements at toxin concentrations from 0.25 mug/mL to 100 mug/mL, it was calculated that the saturable binding component has a Kd of about 2 mug/mL (approx. 60 nM) and a capacity of 0.5 ng per skin disk (1 ng per cm2 of epidermis).

DEVELOPMENTAL TOXICITY

47
Seeley MR, Silbernagel SM, Sweeney C, Faustman EM. EFFECTS OF FOUR ALKYLATING AGENTS ON IN VITRO RAT EMBRYO DIFFERENTIATION. Toxicologist 1992;12(1):333.

The relative developmental toxicity of four direct acting alkylating agents was determined in primary cultures of differentiating rat embryo central nervous system (CNS)

and limb bud (LB) cells and compared with that observed previously in the rat whole embryo post-implantation culture system. The alkylating agents tested in the cell culture system include methylnitrosourea (MNU), ethylnitrosourea (ENU), methylmethanesulfonate (MMS), and ethylmethane-sulfonate (EMS). Cytotoxicity for both CNS and LB was assessed by a neutral red assay. Differentiation of CNS was assessed by haematoxylin staining of neurons; differentiation of LB, by alcian blue staining of extracellular proteoglycans. Relative potencies of these compounds in cell culture was not the same as that observed in the embryo culture system. Whereas rank order of potency in the cell culture system, for cytotoxicity and inhibition of differentiation, was MMS greater than MNU greater than ENU greater than EMS; rank order in the embryo culture system, for embryo lethality and malformations, was MNU greater than ENU greater than MMS greater than EMS. Different mechanisms or pharmacokinetics may be responsible for developmental toxicity in the cell cultures, as compared with the whole embryos.

48

Christian MS. THE EVALUATION OF DEVELOPMENTAL TOXICITY OF CHEMICALS AND OTHER SUBSTANCES. (2) IN VITRO TESTING INCLUDING POSTNATAL EVALUATION. Abstr Pap Am Chem Soc 1991;201(1-2):CHAS 15.

Developmental toxicity risk considerations for humans are 2 of 4 items identified in in vivo developmental toxicity tests: 1) the maternal and developmental no observed adverse effect levels (NOAELs); and 2) the A/D ratio. Three categories of in vitro tests have been proposed to replace mammalian screens: 1) whole embryo culture; 2) organ culture, and 3) cell culture. Most of these proposed tests are inappropriate because: 1) their purpose and outcome were incorrectly identified, due to inconsistent and incorrectly defined terms; 2) most do not validly assess or do not identify the A/D ratio; 3) many are promulgated as systems identifying an agent's potential to produce malformations (exclusive of other types of embryo-fetal toxicity), thus incorrectly predicting "positive" and "negative" teratogenicity, and "validated" on the basis of incorrectly assuming a bimodal mammalian response; or 4) many were evaluated using known developmental toxins but were not examined for multiple classes of agents with specific modes of action. Of the currently used tests, it appears that the Hydra and fish egg developmental toxicity screens have the potential to predict the A/D ratio, while whole embryo, organ and cell culture, including micromass, are most appropriate for examining mechanisms.

49
Gregotti C, Manzo L, Costa LG, Faustman EM. DEVELOPMENTAL TOXICITY OF STYRENE OXIDE IN THE POST-IMPLANTATION EMBRYO CULTURE. Toxicologist 1992; 12(1):333.

The potential of styrene to cause reproductive and developmental disorders in mammals has not yet been fully determined. The aims of these studies were to examine the developmental effects of styrene oxide (SO), the toxic metabolite of styrene, using different in vitro developmental toxicity test systems. In experiments performed with primary embryo cells in culture (micromass system) a 50% reduction of cell differentiation and survival was found to occur at SO concentrations (ug/mL) of 18.6 and 4.4, respectively for CNS cells and 7.2 and 28.2 ug/mL for limb bud (LB) cells. The differences in concentrations of SO affecting cell viability versus differentiation for CNS suggests that inhibition of differentiation by SO is a reflection of SO cytotoxicity. The results also indicated a selective sensitivity of mesenchymal cells to SO in the differentiation process. In a separate series of studies, the post-implantation whole embryo culture (WEC) system was used to examine the embryotoxic effects of SO. Endpoints of developmental toxicity monitored in these studies included embryolethality, malformation, growth retardation and changes in macromolecular cell components following SO exposures. SO induced a dose dependent increase in embryolethality and embryo malformation at concentrations that were comparable to effective concentrations in the micromass culture system. These findings suggest quantitative comparability between two in vitro developmental toxicity systems.

50
Whittaker SG, Faustman EM. EFFECTS OF BENZIMIDAZOLE ANALOGS ON CULTURES OF DIFFERENTIATING RODENT EMBRYONIC CELLS. Toxicol Appl Pharmacol 1992; 113(1):144-51.

Micromass cell culture systems for rat embryo midbrain (CNS) and limb bud (LB) cells were employed to assess the in vitro developmental toxicity of the benzimidazole analogs, mebendazole MBZ), thiabendazole (TBZ), and nocodazole (NCZ), in addition to the classic microtubule inhibitor, colchicine. Comparison was made to albendazole (ABZ), a previously studied benzimidazole anthelmintic. Two parameters for assessing developmental toxicity were measured: differentiation and cytotoxicity. The relative

potencies of the benzimidazole analogs in the micromass system (NCZ greater than MBZ; ABZ much greater than TBZ) mirrored their effectiveness in an assay for in vitro inhibition of mammalian tubulin polymerization. Colchicine also exhibits a high affinity for mammalian tubulin and was a potent inhibitor of cell proliferation, chondrogenesis, and neuronal differentiation. With the exception of TBZ, these agents should be considered potential developmental toxicants since they inhibit cell growth and differentiation of micromass cultures at nanomolar concentrations.

EMBRYOTOXICITY

51

Kemppainen BW, Terse P, Madhyastha MS, Zurovac O, Stringfellow D. PRELIMINARY COMPARISON OF THE RELATIVE SENSITIVITY OF CULTURED EMBRYONIC TISSUES/CELLS TO TERATOGENIC AGENTS. Teratology 1992;45(5):506-7.

Assays were developed to compare the relative sensitivity of in vitro cultured murine pre-implantation embryos, primary cultures of human amniotic fluid cells, and a continuous cell line derived from bovine trophectoderm (BE12-6) for detection of teratogenic agents. Pre-implantation embryos collected from super-ovulated mice were cultured for 72 hours in the presence of 10-fold dilutions of the test compounds. The embryos that hatched and attached at the end of the culture period were considered normal. The embryonic cells were seeded in 96-well plates, cultured for 24 hours in control media, exposed to 10- and 2-fold dilutions of the test compounds for 72 hours, and finally were stained and counted. IC50, the concentration (mg/mL) which inhibited embryonic development or cell growth by 50%, was calculated for 12 compounds. Results indicate that sensitivity of culture systems increases from human amniotic fluid cells less than BE12-6 cells less than murine pre-implantation embryos. Results of additional compounds will be presented.

52

Eto K, Shiota K. THE STUDY OF CONGENITAL ANOMALIES IN CULTURE OF CELLS AND WHOLE EMBRYOS. Teratology 1991; 44(6):5B.

Culture techniques of cell, tissue, organ and whole embryo are practical tools for the elucidation of mechanism in congenital anomalies in vitro. In the described workshop, studies using micromass culture of limb bud and midbrain cells of rodent embryos, and also whole embryo culture during the period of preimplantation, organogenesis and post

organogenesis to term stages are introduced. Cell differentiation may be efficiently examined in micromass culture, however, available cell types are a few such as limb bud, midbrain and facial mesenchymal cells. The advantages of whole embryo culture are: 1) precise control of conditions (concentration of administrating chemical or biological agents, duration of exposure, site of exposure in embryo or membranes and stage of embryonic development), 2) detection of metabolic activity of embryo by sampling the medium, gas phase of culturing tissues, 3) continuous observation of culturing embryos with the possibility of microsurgery and microinjection. However, culture techniques also have disadvantages such as 1) very exacting and laborious, 2) limited culture periods, 3) undetachable functional abnormalities and so forth. The limitations and possibilities of these culture techniques in practical experiments, and the combined method of cell and whole embryo culture, cell and organ culture or in vivo and in vitro method are discussed.

53
Natarajan AT, Duivenvoorden WC M, Meijers M, Zwanenburg TS B. INDUCTION OF MITOTIC ANEUPLOIDY USING CHINESE HAMSTER PRIMARY EMBRYONIC CELLS. TEST RESULTS OF 10 CHEMICALS. Mutat Res;287(1):47-56.

Using primary Chinese hamster embryonic cells, 10 known or suspected aneugens supplied as a part of the EC 4th Environmental Research and Development Program were evaluated by the technique described by F.N. Dulout and A.T. Natarajan (1987). The chemicals included cadmium chloride, chloral hydrate, colchicine, diazepam, econazole, hydroquinone, pyrimethamine, thiabendazole, thimerosal and vincristine. All chemicals except pyrimethamine gave clearly positive effect at most the the doses tested. The ease with which the assay is performed and reproducible results that are obtained with the suspected compounds indicate that this in vitro test using primary embryonic fibroblasts is a promising one for routine screening.

GENOTOXICITY

54
Ignatius A, Hund M, Tempel K. POLY(ADP-RIBOSE)POLYMERASE-ACTIVITY OF CHICKEN EMBRYO CELLS EXPOSED TO NUCLEOTOXIC AGENTS. Toxicology 1992;76(2):187-196. (34 REFS)

The use of an assay measuring poly(ADP-ribose)polymerase (PARP) activity in chick embryo cells in genotoxicity studies was examined. Cell cultures were prepared from brain

and liver cells isolated from 15 day old white-leghorn-chicken embryos. Comparisons were made with thymic cells isolated from Sprague-Dawley-rats. The cultures were treated with X-rays, ultraviolet irradiation, or various chemicals. PARP activity was determined by measuring the incorporation of the ADP-ribose moiety of carbon-14 labeled NAD into acid insoluble precipitates. The authors conclude that the measurement of PARP activity in chick embryo cells is useful in the determination of nucleotoxicity.

55
Myhr BC, Lawlor TE, Young RR, Murli H, Cifone MA. GENOTOXICITY ASSESSMENT OF PERFLUORODECANOIC ACID USING A BATTERY OF IN VITRO AND IN VIVO/IN VITRO ASSAYS. Report; ISS AAMRL-TR-90-070; Order No. AD-A240490,, 1990,80 pp. NTIS Springfield, VA, USA.

Perfluoro-n-decanoic acid (PFDA), a perfluorinated fatty acid was evaluated in in vitro bioassays to assess its potential genotoxic activity. The assays conducted were the Ames Salmonella/microsomal mutagenicity assay, the CHO gene mutation assay, the CHO/sister chromatid exchange (SCE) and chromosome aberrations assays, the BALB/c-3T3 cell transformation assay and in vivo/in vitro unscheduled DNA (UDS) and S-phase synthesis assays. The results of the genetic tests indicated that PFDA does not interact with genetic material except possibly at highly toxic doses. No responses were found in the Ames assay, SCE assay, and cell transformation assay. However, in the chromosomal aberrations assay highly toxic treatments of cells in culture with PFDA induced a dose-related increased in chromosomal aberrations in the presence of S9 microsomal enzymes. Responses obtained indicate the rapid onset of a low-level hepatotoxicity but the genetic test battery results predicted that little or no genetic risk would occur from exposure to PFDA.

56
Parry JM. AN EVALUATION OF THE USE OF IN VITRO TUBULIN POLYMERISATION, FUNGAL AND WHEAT ASSAYS TO DETECT THE ACTIVITY POTENTIAL CHEMICAL ANEUGENS. Mutat Res 1993; 287(1):23-28.

The test chemicals included in the EC Aneuploidy Project were evaluated for their ability to induce aneuploidy or aneuploidy related endpoints in assays using in vitro tubulin polymerisation, fungi and wheat. The results obtained demonstrated considerable qualitative and quantitative differences between the responses of the assays

to the 10 test chemicals. Fungal assays failed to respond to the potent mammalian spindle poisons colchicine and vinblastine and only three chemicals were positive in all three fungal test systems i.e. chloral hydrate, thimerosol and thiabendazole. The in vitro tubulin polymerisation assays produced unambiguous positive results with three chemicals i.e. colchicine, thimerosol and vinblastine sulphate. The hexaploid wheat assay produced a positive

response with 8 of the test chemicals i.e. colchicine, econazole, thimerosol, pyrimethamine, thiabendazole, cadmium chloride, vinblastine and diazepam. However, the wheat assay was relatively insensitive to the potent spindle poison colchicine.

57
Bernard BA, Bernardon JM, Delescluse C, Martin B, Lenoir MC, Maignan J, Charpentier B, Pilgrim WR, Reichert U, Shroot B. IDENTIFICATION OF SYNTHETIC RETINOIDS WITH SELECTIVITY FOR HUMAN NUCLEAR RETINOIC ACID RECEPTOR GAMMA. Biochem Biophys Res Commun 1992; 186(2):977-83.

The action of retinoids on gene regulation is mediated by three distinct nuclear retinoic acid receptor (RAR) subtypes called RAR alpha, beta and gamma. Since RAR gamma is predominantly expressed in adult skin, specific ligands for this subtype could (i) represent valuable tools to evaluate the biological role of RAR gamma in skin and (ii) provide therapeutic entities with a higher therapeutic index at lower teratogenic risk. Using in vitro binding studies and a functional transactivation assay, we have identified three compounds with high RAR gamma selectivity.

58
Kindig D, Garriott ML, Parton JW, Brunny JD, Beyers JE. DIPHENYLHYDANTOIN IS NOT GENOTOXIC IN A BATTERY OF SHORT-TERM CYTOGENETIC ASSAYS. Teratogenesis Carcinog Mutagen 1992; 12(1):43-50.

5,5-Diphenylhydantoin (DPH) is an antiepileptic drug associated with an increase in malformations in infants born to women taking DPH during pregnancy. Positive and negative results have been reported by various investigators for in vivo and in vitro chromosome aberration (CAB) assays, in vivo and in vitro sister chromatid exchange (SCE) assays, and in vivo micronucleus tests (MNT). In the author's laboratory, DPH was tested in an in vitro CAB assay using Chinese hamster ovary cells with and without an S-9 activation system, an in vivo SCE assay in female CD-1 mice,

an in vivo MNT, using both male and female CD-1 mice, and a transplacental micronucleus test. The results from this comprehensive battery of cytogenetic tests were uniformly negative and support a conclusion that the known teratogen, DPH, is not clastogenic.

59
Ma T-H, Sandhu SS, Peng Y, Chen TD, Kim T. SYNERGISTIC AND ANTAGONISTIC EFFECTS ON GENOTOXICITY OF CHEMICALS COMMONLY FOUND IN HAZARDOUS WASTE SITES. Mutat Res 1992; 270(1):71-77. (20 REFS)

The genotoxicity of mixtures of lead-tetraacetate (LTA), arsenic-trioxide, dieldrin, and tetrachloro- ethylene was investigated to examine possible synergistic and antagonistic effects on the genotoxicity of compounds typically found as part of mixtures at hazardous waste sites. The findings indicate that when assessing the biological risks from chemical exposures at hazardous waste sites, the genotoxicity of their mixtures must be evaluated, since the compounds may behave differently in a mixture than when alone.

60
Vian L, Bichet N, Gouy D. THE IN VITRO MICRONUCLEUS TEST ON ISOLATED HUMAN LYMPHOCYTES. Mutat Res 1993; 291(1):93-102.

The in vitro micronucleus test was performed on isolated human lymphocytes using the cytokinesis-block technique with and without a rat liver metabolizing system. Positive control substances were used to evaluate this test: a direct agent (vincristine) requiring no metabolic activation, and three promutagens (cyclophosphamide, benzo[a]pyrene and dimethylbenz[a]anthracene). All of them, when compared with controls, caused a significant increase in micronucleus frequency, with a clear dose response. Five compounds were then tested in this in vitro micronucleus test: safrole, azathioprine, procarbazine, ethylstilbestrol and o-toluidine. The chemicals were examined with and without exogenous metabolic activation. Of these five compounds, o-toluidine was a marked direct genotoxic agent and azathioprine gave positive results with or without metabolic activation (a better response was noted without the addition of S9 mix). Diethylstilbestrol gave conflicting results and was considered inconclusive. Two chemicals, safrole and procarbazine, were nongenotoxic in this test system, whatever the protocol used.

61
Godin CS, Myhr C, Lawlor TE, Young RR, Murli H.
GENOTOXICITY ASSESSMENT OF CHLOROTRIFLUOROETHYLENE TETRAMER
ACID USING A BATTERY OF IN VITRO AND IN VIVO/IN VITRO
ASSAYS. Report; ISS AAMRL-TR-90-069; Order No. AD-A240492,
1990,77 pp. NTIS Springfield, VA, USA.

Chlorotrifluoroethylene (CTFE) tetramer acid, the
perhalogenated fatty acid metabolite of the eight-carbon
oligomer of polychloro-trifluoroethylene (pCTFE), was
evaluated in in vitro bioassays to assess its potential
genotoxic activity. The assays conducted were the Ames
Salmonella/microsomal mutagenicity assay, the CHO gene
mutation assay, the CHO/sister chromatid exchange
and chromosome aberration assays, the BALB/c-3T3 cell
transformation assay and in vivo/in vitro unscheduled
DNA (UDS) and S-phase synthesis assays. CTFE tetramer acid
did not demonstrate genotoxic potential in any of the in
vitro assays and was also negative in the UDS assay.
However, a small, but significant, dose-related increase in
S-phase synthesis occurred 48 h following oral
administration of doses of CTFE tetramer and ranging from 11
to 44 mg/kg. This increase in S-phase synthesis indicated a
rapid response to hepatotoxicity caused by CTFE tetramer
acid but the results of the test battery would predict no
genetic risk from exposure.

62
McCann J, Dietrich F, Rafferty C, Martin AO. A CRITICAL
REVIEW OF THE GENOTOXIC POTENTIAL OF ELECTRIC AND MAGNETIC
FIELDS. Mutat Res 1993; 297(1):61-95. (86 REFS)

Fifty-five published articles were identified which
reported results of tests of ELF (extremely low frequency)
or static electric or magnetic fields for genotoxic effects.
The biological assays used spanned a wide range, including
microbial systems, plants, Drosophila, mammalian and human
cells in vitro and in vivo. Experimental results were
grouped into four exposure categories: ELF Electric; ELF
Magnetic; Static Electric; and Static Magnetic. All
experiments were critically analyzed with respect to basic
data quality criteria. Experiments within each exposure
category were then compared to determine if results
reinforced or contradicted one another. The preponderance of
evidence suggests that neither ELF nor static electric or
magnetic fields have a clearly demonstrated potential to
cause genotoxic effects. However, there may be genotoxic
activity from exposure under conditions where phenomena
auxiliary to an electric field, such as spark discharges,

electrical shocks, or corona can occur. In addition, two unconfirmed reports suggest the genotoxic potential of certain chemical mutagens or ionizing radiation may be affected by co-exposure to electric or magnetic fields. Certain exposure categories are not represented or are under-represented by tests in some genotoxicity test systems that are usually included in minimal test batteries as specified by EPA for chemicals. It is suggested that consideration be given to whether additional genotoxicity testing is warranted to fill experimental gaps.

63
Parodi S, Malacarne D, Taningher M. EXAMPLES OF USES OF DATABASES FOR QUANTITATIVE AND QUALITATIVE CORRELATION STUDIES BETWEEN GENOTOXICITY AND CARCINOGENICITY. Environmental Health Perspectives 1991; 96:61-66. (24 REFS)

Some examples are presented of using genotoxicity and carcinogenicity databases for quantitative and qualitative correlation studies between short-term tests and carcinogenicity. Database quality is obviously important but quantity is as well: one of the major deficiencies of present databases is that they are too small. Using relatively small, different databases, different results can be obtained. With small databases it is difficult to disaggregate data for homogeneous chemicals classes or other types of subsets. Using the databases of Gold (carcinogenicity) and Wurgler (genotoxicity), the carcinogenic potency of genotoxic and nongenotoxic carcinogens for different chemical classes has been investigated.

64
Gollapudi BB, Linscombe VA, McClintock ML, Sinha AK, Stack CR. TOXICOLOGY OF DIETHYLENE GLYCOL BUTYL ETHER: 3. GENOTOXICITY EVALUATION IN AN IN VITRO GENE MUTATION ASSAY AND AN IN VIVO CYTOGENETIC TEST. J Am Coll Toxicol 1993; 12(2):155-159.

DGBE was evaluated in a forward gene mutation assay at the HGPRT locus of CHO cells in culture and in an in vivo mouse bone marrow micronucleus test for cytogenetic damage. DGBE did not elicit a positive response in the CHO/HGPRT assay when tested up to a maximum concentration of 5000 mug/ml with and without an external metabolic activation system (S-9). In the micronucleus test employing three post-treatment bone marrow sampling times (24, 48, and 72 hr), DGBE was ineffective in increasing the incidence of micronucleated polychromatic erythrocytes (MN-PCE)

when tested in both sexes up to a maximum tolerated dose of
3300 mg/kg body weight. Thus, these data and those of others
indicate a general lack of genotoxic potential for DGBE in
short-term tests.

65
Madle S, Mueller L. TESTING STRATEGIES IN GENETIC
TOXICOLOGY. BGA Schr.; VOL 1/93, 1993,7-11.

No abstract.

66
Whong WZ. DEVELOPMENT OF A LUNG-CELL MODEL FOR
STUDYING WORKPLACE GENOTOXICANTS. Report; ISS Order No.
PB92-114644,1991:25. NTIS, Springfield, VA, USA.

The objectives of this study were to establish in vivo
and/or in vitro multiple genetic endpoint assay systems
using lung cells of the rat, to compare the sensitivity of
rat lung cells to genotoxicants between in vivo and in vitro
assay systems, and to evaluate the suitability of the
multiple genetic endpoint/lung cell assay system for
detecting genotoxicity. Male CD-rats were used in the study.
The best enzymic sepn. of rat lung cells was a combined
treatment of lung with trypsin and collagenase or a cold
digestion with protease. Two mug cytochalasin B/mL for 2
days of incubation was the optimal protocol for cytokinesis
block in lung cells during the micronucleus formation (MN)
assay. Primary lung cells can be used for in vivo and in
vitro sister chromatid exchange and MN analyses. A minimal
16 h cell incubation in the presence of tritium-labeled
thymidine was required for visualization of optimal DNA
repair in the lung cell/unscheduled DNA synthesis (UDS)
assay system. Both alveolar macrophages and primary lung
cells could be used for in vivo and in vitro UDS assays.

67
Endo-Capron S, Renier A, Janson X, Kheuang L, Jaurand
MC. IN VITRO RESPONSE OF RAT PLEURAL MESOTHELIAL CELLS TO
TALC SAMPLES IN GENOTOXICITY ASSAYS (SISTER CHROMATID
EXCHANGES AND DNA REPAIR). Toxicol In Vitro 1993; 7(1):7-14.

The genotoxicity of three samples of talc has been
determined using in vitro cell systems previously developed
for testing asbestos fibres. The talc samples used consisted
of particles of respirable size in order to test the effect
of particles likely to be deposited in the lung.
Genotoxicity was tested in cultures of rat pleural

mesothelial cells (RPMC) using genotoxicity assays for unscheduled DNA synthesis (UDS) and sister chromatid exchanges (SCEs). The effects were compared with those obtained with negative controls (attapulgite and anatase) and positive controls (chrysotile and crocidolite asbestos). In contrast to asbestos, none of the talc samples, nor the negative controls, induced enhancement of UDS or SCEs in treated cultures in comparison with the untreated cultures.

HEPATOTOXICITY

68

Hall TJ, James PR, Cambridge G. DEVELOPMENT OF AN IN VITRO HEPATOTOXICITY ASSAY FOR ASSESSING THE EFFECTS OF CHRONIC DRUG EXPOSURE. Res Commun Chem Pathol Pharmacol 1993;79(2):249-56.

We have used the human hepatoma cell line HepG2 to compare the hepatotoxic effects of acute (24 hr) and chronic (up to 10 days) exposure to amitriptyline, paracetamol and ondansetron. In acute exposure studies, hepatotoxicity was assessed by the sulforhodamine B protein staining method,

where amitriptyline and paracetamol produced 50% hepatotoxicity at concentration of 30 microM and 7 mM, respectively, while ondansetron was non-hepatotoxic at 100 microM, the highest concentration used. In chronic exposure studies, the morphology of HepG2 cells was assessed by phase microscopy every 2 days and the compounds, at concentrations determined from the acute assay, were added fresh every 2 days. Chronic exposure to amitriptyline and paracetamol produced significant morphological changes in HepG2 cells at 3 microM and 1 mM respectively, concentrations which had no significant effect in the acute assay. Ondansetron (100 microM) produced only slight morphological changes in the cells after 10 days of culture. The combination of acute and chronic drug exposure assays with HepG2 cells represents novel in vitro systems for the hepatotoxicological assessment of drugs intended for human use.

69

Bando Y, Kunitomo K, Komi N. AN IN VITRO CHEMOSENSITIVITY TESTING SYSTEM FOR CYCLOPHOSPHAMIDE USING PRIMARY CULTURED PARENCHYMAL RAT HEPATOCYTES. J Jpn Soc Cancer Ther 1993;28(1):58-70.

An in vitro chemosensitivity testing system for cyclophosphamide (CPA) was developed using cultured parenchymal rat hepatocytes on collagen coated microcarrier beads. Isolation of hepatocytes was accomplished by the

perfusion of a collagenase solution. While cultivating tumor cells with hepatocytes, both cells were separated by a microporous membrane in RPMI-1640 which contained 10% fetal bovine serum and several doses of CPA for a three day period. The inhibition index (I.I.) was calculated by measuring intracellular ATP levels of tumor cells (ATP assay). Tumor cells were determined to be sensitive when I. I. was more than 50%. As a result of in vitro testing, AH-13 and AH-130 cell lines were sensitive and AH-109A cell line was resistant to CPA. Significant elongation of survival was observed in AH-13 and AH-130 inoculated rats, but not in AH-109A inoculated rats. The results indicate in vitro testing predict the results of in vivo treatment and made evident that cultured hepatocytes can activate CPA. This system may be useful for in vitro testing for other masked compounds and for use in clinical situations.

IMMUNOTOXICITY

70

Hersey P, Magrath H, Wilkinson F. DEVELOPMENT OF AN IN VITRO SYSTEM FOR THE ANALYSIS OF ULTRAVIOLET RADIATION-INDUCED SUPPRESSION OF NATURAL KILLER CELL ACTIVITY. Photochem Photobiol 1993; 57(2):279-84.

Previous studies have shown that natural killer (NK) cell activity was suppressed in volunteer subjects exposed to ultraviolet radiation (UVR) from solarium lamps. The present studies were carried out to determine the spectrum of UVR responsible for suppression of NK activity and to develop in vitro methods to analyze the effectiveness of sunscreen agents in prevention of UVR-mediated suppression of NK activity and other aspects of immune function. UVR from a xenon arc lamp source was used to irradiate peripheral blood lymphocytes (PBL) in wells of tissue culture flasks, and transmission interference filters were used to eliminate UVR of particular wavelengths. The results indicated that UVR from this source inhibited NK activity of PBL in a dose-dependent manner with a 50% inhibitory dose of 5.5 mJ/cm2 when unfiltered and 29.6 mJ/cm2 when diluted through cellulose acetate, which gave a UV spectrum similar to that in solar radiation. Equivalent suppression of NK activity was mediated by UV-A (UVR > 315 nm) at dose levels of 4.2 J/cm2, which was approximately 140 times greater than the amount of UV-B (UVR < 315 nm) needed to suppress NK activity. These studies suggest that when the greater proportion of UV-A in solar radiation and its greater penetration into skin is taken into account, UV-A may have equivalent or greater direct immunosuppressive effects than UV-B.

71
Nagata S, Masuda K, Nogusa H, Hirano K, Takagishi Y.
NEW APPLICATION OF HUMAN TUMOR CLONOGENIC ASSAY TO IN VITRO
EVALUATION OF TUMOR-TARGETING EFFICIENCY OF
IMMUNOCONJUGATES. Chem Pharm Bull 1992; 40(8):2151-4.

This report proposes an efficient in vitro method for the evaluation of drug targeting with monoclonal antibody as a carrier to tumor cells. Monoclonal antibody (35G; IgG2a) selectively binding to alpha-fetoprotein (AFP) from human hepatoma cells (HuH-7) was conjugated with an anticancer drug, vindesine (VDS). Human tumor clonogenic assay (HTCA) with some modifications was applied to estimate the targeting efficiency of a conjugate (VDS-35G) for the first time. HTCA was shown to be a useful in vitro evaluation method for drug targeting.

72
Mori S, Nishimura N, Nakamura T, Masuda M, Oba K. THE
LYMPHOCYTE PROLIFERATION ASSAY AS AN IN VITRO ALTERNATIVE
METHOD TO SENSITIZATION TESTS. In Vitro Toxic - J Molec
Cell Toxic 1992; 5(3):147-160. (14 REFS)

An in-vitro lymphocyte proliferation assay for detecting skin sensitizers was developed. BALB/c-mice were immunized by application of 200 microliters (microl) of the test compounds (0.5 to 2%) to the shaved abdomen. Five days later, the axillary and inguinal lymph nodes were removed, placed in balanced salt solution (BSS), and teased apart to obtain single cell lymphocyte suspensions. The lymphocytes were washed twice with BSS and then placed in a culture medium containing 10% heat inactivated horse serum and fresh mouse serum. The cell suspensions were added to microculture plates, each well containing 0.2 microcurie of tritium labeled thymidine. A 20 microl solution of the test compound was added to the suspensions. Lymphocyte proliferation responses to the test compound were determined by measuring uptake of tritiated thymidine after 5 days of incubation. The sensitizing potential of the test compound was assessed by determining the stimulation index (SI), the ratio of tritium activity in the cells in the presence of the test compound to the activity in the absence of the compound. The assay was tested using the strong water soluble and insoluble sensitizers. The assay readily detected the strong sensitizers, yielding SIs of 2.0 to 32.7. The weak sensitizers were also easily detected, producing SIs of 1.8 to 14.1. The authors conclude that the assay can readily detect the antigenicity of sensitizers of varying strength regardless of their

solubility. It can be used to screen the ingredients of new products for their sensitizing potential before the product is developed.

73
Jeney G, Anderson DP. AN IN VITRO TECHNIQUE FOR SURVEYING IMMUNOSTIMULANTS IN FISH. Aquaculture 1993; 112(2-3):283-7.

In vitro assays were developed to investigate the effects of immunostimulants on leukocytes in spleen sections of rainbow trout (Oncorhynchus mykiss). Fish spleens were divided, and individual sections were placed in 10 mL of tissue culture media and 0.1, 1, 10, or 100 mug/mL dilutions of levamisole, QAC (quaternary ammonium compound), or ISK (a polypeptide). After 4 days of incubation, cell suspensions were prepared to examine the effects of the immunostimulants on neutrophil oxidative activity using the nitroblue tetrazolium (NBT) assay and on phagocytic activity by engulfment of glutaraldehyde-fixed sheep red blood cells. QAC induced a heightened activity in plates receiving 0.1 and 1.0 mug/mL, but suppressed activity at 10 and 100 mug/mL levels. Sections incubated with ISK showed heightened responses at all doses except 100 mug/mL. Levamisole showed heightened responses at all doses in the NBT assay, and at the 1.0 and 10 mug/mL doses in the phagocytic assay.

74
Lachapelle M, Guertin F, Marion M, Fournier M, Denizeau F. MERCURIC CHLORIDE AFFECTS PROTEIN SECRETION IN RAT PRIMARY HEPATOCYTE CULTURES: A BIOCHEMICAL ULTRASTRUCTURAL, AND GOLD IMMUNOCYTOCHEMICAL STUDY. J Toxicol Environ Health 1993;38(4):343-354.

The toxicity of mercury on hepatocytes was studied at the ultrastructural, biochemical, and immunocytochemical levels. Albumin metabolism was examined because it is a representative liver-specific function. A novel cytochemical method using the protein A-gold technique for the in situ localization of albumin in hepatocye cultures was applied. Primary rat hepatocyte cultures were exposed to increasing HgCl2 concentrations. Cytotoxicity was assessed by measuring the release of lactic dehyrogenase from the cells. At the highest exposure concentration tested (50 muM), Hg was found to be significantly cytotoxic in contrast to what occurred at 5.0 and 0.5 muM. The level of albumin secreted, as measured by ELISA, was decreased by approximately 38% at 5.0 muM HgCl2 and was found not to be different from that of

controls at lower concentrations. Immunocytochemical detection of albumin-immunoreactive sites using protein A-gold labeling further revealed that these were less abundant in hepatocytes treated with 5.0 muM HgCl2(-64%) as compared to control preparations. The results suggest that one of the effects of mercury on hepatocytes is to affect liver-specific functions such as albumin production, possibly through interference with ribosomal function. This study demonstrated for the first time the applicability of the high-resolution protein A-gold technique for toxicological investigations using hepatocytes in vitro.

75
Padgett EL, Barnes DB, Pruett SB. DISPARATE EFFECTS OF REPRESENTATIVE DITHIOCARBAMATES ON SELECTED IMMUNOLOGICAL PARAMETERS IN VIVO AND CELL SURVIVAL IN VITRO IN FEMALE B6C3F1 MICE. J Toxic Environ Health 1992; 37(4):559-571.
(14 REFS)

The immunotoxicity of sodium-methyldithiocarbamate (SMD), disodium-ethylene-bis (dithiocarbamate)(EBD), and sodium-diethyldithiocarbamate (DEDTC) was studied in mice. Female B6C3F1-mice were gavaged with SMD, EBD, or DEDTC daily for 7 days. They were killed 24 hours after the last dose and weighed. The spleen and thymus were removed and weighed. Splenocytes were harvested and splenic natural killer (NK) cell activity against YAC-1 tumor cells was measured. Splenocytes and thymocytes collected from untreated mice were cultured and incubated with up to 50.0 micromolar (microM) SMD, EBD, or DEDTC for 20 hours. Cytotoxicity was assessed by determining viability using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium-bromide dye reduction assay. All compounds were cytotoxic to thymocytes and splenocytes in-vitro. SMD, EBD, and DEDTC concentrations causing 50% cell death (EC50s) for splenocytes were 9.5, 10.6, and 0.13microM, respectively. The corresponding EC50s for thymocytes were 6.6, 9.8, and 0.15microM. The authors conclude that the in-vivo immunotoxic potential of SMD as measured by its effect on thymus and spleen weight and splenic NK cell activity is much greater than that of EBD and DEDTC. The in-vitro cytotoxic potency of SMD, EBD, and DEDTC does not correlate with their in-vivo potency. Direct cytotoxicity does not appear to be a mechanism by which SMD, EBD, and DEDTC cause immunological changes in-vivo.

76
Moore KG, Dannenberg AM Jr. ANTIGEN-SPECIFIC IGG1-MEDIATED EPIDERMAL CELL INJURY: A COMPONENT OF CONTACT

HYPERSENSITIVITY REACTIONS IN GUINEA PIGS, MEASURABLE IN VITRO IN FULL-THICKNESS SKIN EXPLANTS. J Invest Derm 1992; 98(6):929-935. (50 REFS)

A technique for studying components of the allergic contact sensitivity reaction was described. Female Hartley-guinea-pigs were sensitized on days one through three with 500 micrograms 2,4-dinitro-1-chlorobenzene (DNCB) or 4-ethoxymethylene-2-phenyl-oxazolone (oxazolone). The chemicals were used on the caudal part of the back and were reapplied on day ten. After 17 days, the animals were sacrificed and blood sera samples were collected. Skin explants of 1 square centimeter were obtained from unsensitized guinea-pigs and used for evaluation of vacuoles, mast cells, and microblistering as well as radiolabeled leucine incorporation. Sera samples were used for purification of immunoglobulin-G and immunoglobulin-G1 (IgG1). Results indicated a strong antibody/antigen response when guinea-pigs receive a second exposure to DNCB or oxazolone. The response also occurred when skin explants underwent secondary exposure in-vitro. The authors concluded that several contact sensitivity reaction components can be recreated in-vitro through transfer of sera containing antigen specific immunoglobulins. Sera banks could be helpful for the identification of preexisting sensitivities to chemical compounds in humans.

77

Henningsen GM. DERMAL HYPERSENSITIVITY: IMMUNOLOGIC PRINCIPLES AND CURRENT METHODS OF ASSESSMENT IN DERMAL AND OCULAR TOXICOLOGY: FUNDAMENTALS AND METHODS. D. W. Hobson, Editor; CRC Press, Boca Raton, Florida, 1991;153-192. (221 REFS)

Immunologic principles and methods for investigating dermal hypersensitivity were reviewed. The epidemiological aspects of chemically allergic contact dermatitis were summarized. Estimates by various governmental agencies and groups have indicated that skin problems account for up to 28% of reported illnesses. NIOSH considers dermatological conditions to be one of the ten leading occupational diseases. Allergic contact dermatitis has been estimated to account for 20 to 25% of all cases of occupational dermatitis. The skin has been characterized as a secondary immune organ in terms of innate resistance to infections and acquired specific immunity to foreign antigens. The immunological functions of the skin as they pertain to disease resistance and immunopathology were considered. Individual variations in the expression of allergic contact dermatitis were described. The immunologic basis of dermal

hypersensitivity was considered. The mechanisms of allergic contact dermatitis and atopic dermatitis were discussed. The effects of immune system dysfunction induced by biomolecules, drugs, chemicals, and immunomodulatory physical agents and diseases on dermal hypersensitivity were considered. Current methods for assessing dermal hypersensitivity were reviewed. In-vitro screening methods for assessing dermal hypersensitivity were summarized.

MECHANISMS

78

Ponath PD, Farr SB. NOVEL IN-VITRO ASSAYS FOR ELUCIDATING MOLECULAR MECHANISMS OF TOXICITY. Keystone Symposium on Discovery and Development of Therapeutic Compounds, Snowmass, Colorado, USA, MARCH 20-26, 1993. J Cell Biochem Suppl 1993; 0 (17 PART D):177.

No abstract.

79

Lee T-P. HUMAN LEUKOCYTES AS AN IN VITRO MODEL FOR STUDYING PCB TOXICITY in In Vitro Methods of Toxicology, R. R. Watson, Editor; CRC Press, Boca Raton, Florida, 1992; 163-173. (97 REFS)

The use of in-vitro studies to elucidate the biochemical mechanisms involved in the effects of polychlorinated biphenyls (PCBs) on leukocyte activity was examined. A review of the effects of PCB exposure on the immune system and other organ systems was presented. Methods used for studying the effects of PCBs on leukocyte cell cultures were described. These included the separation of cell populations from whole peripheral blood by centrifugation and the assessment of chemical toxicity by examining various cell activities and cell viability. Tests for the evaluation of lymphocyte function were described such as the mitogenic response, rosette formation with sheep red blood cells, the removal and regeneration of receptors for rosettes, and lymphokine secretions. Tests for the evaluation of neutrophil function have included chemotactic responses, lysosomal enzyme release, phagocytic activity, oxygen consumption, and assessment of the phagocytic and cytotoxic activities in response to Staphylococcus.

METABOLISM/XENOBIOTICS

80

Voss JU, Seibert H. MICROCARRIER-ATTACHED RAT HEPATOCYTES

AS A XENOBIOTIC-METABOLIZING SYSTEM IN COCULTURES. Cell Biol Toxicol; 7(4):387-99.

A method for the primary culture of rat liver cells on collagen-coated dextran microcarriers is described. Ethoxycoumarin deethylase (EOD) activity 24 h after inoculation was comparable for liver cells cultured on microcarriers and on collagen-coated dishes. Cells were cultured on microcarriers for up to 48 h and retained 25% of the initial EOD activity that was seen in freshly isolated liver cells. Microcarrier-attached hepatocytes were cocultured with BALB/c 3T3 cells to study the metabolism-mediated cytotoxicity of cyclophosphamide (CPA). In the absence of hepatocytes, growth of 3T3 cells was not affected by CPA at concentrations up to 3600 .mu.M. In coculture with hepatocytes, cytotoxicity of CPA was expressed in a time- and concentration-dependent manner. At high concns., CPA slightly depressed the EOD-activity of hepatocytes. These results indicate that cocultivation of microcarrier-attached rat liver cells with target cells represents a valuable approach to the study of the metabolism-mediated toxicity of xenobiotics in vitro.

81

Darroudi F, Natarajan AT. METABOLIC ACTIVATION OF CHEMICALS TO MUTAGENIC CARCINOGENS BY HUMAN HEPATOMA MICROSOMAL EXTRACTS IN CHINESE HAMSTER OVARY CELLS (IN VITRO). Mutagenesis 1993;8(1):11-5.

The efficiency of human hepatoma (Hep G2) S9 microsomal fractions to activate indirectly acting genotoxic carcinogens was evaluated. The extract was prepared from Hep G2 epithelial cells, following sonication and centrifugation. The mutagenic activity of cyclophosphamide, benzo[a]pyrene, pyrene, hexamethylphosphoramide and safrole was assessed by the ability of their activated metabolites to induce sister chromatid exchange (SCE) and micronuclei (MN) in Chinese hamster ovary cells (CHO) (treated in vitro). All promutagenic carcinogens tested were found to be effective only following metabolic activation by Hep G2 cell extracts. Non-carcinogen pyrene was not able to induce an increase in the frequencies of SCE or MN in CHO cells even in the presence of Hep G2 S9 microsomal fractions. Parallel experiments were carried out using rat liver homogenate (S9 fraction) as an exogenous activation system, and comparisons were made between these two in vitro systems and in vivo assays using the rodent.

82

Oesch F, Oesch-Bartlomowicz B, Arens HJ, Friedberg T, Utesch D, Glatt HR, Platt KL. MOLECULAR AND CELLULAR BASIS FOR ADEQUATE METABOLIC DESIGN OF GENOTOXICITY STUDIES. Toxicology Letters 1992, 64-65:643-649. (2 REFS)

The adequacy of metabolic techniques for the evaluation of genotoxicity was reviewed in terms of molecular and cellular aspects. Most genotoxic compounds were not active in themselves and were metabolized to reactive products by the action of complex activating and precursor sequestering systems which differed greatly between species of animals, humans, and the various test systems. In exogenous test systems, the post mitochondrial supernatant fraction of liver homogenate and a cofactor generating complex were added, which was somewhat artificial. Unfavorable conditions for conjugating reactions in in-vitro tests with subcellular metabolizing enzymes impaired the quantitative correlation of mutagenicity with animal carcinogenicity by distorting the metabolic situations. Questions raised included the validity of results which did not take into account aspects such as dilution of cofactors, and high dose carcinogenicity induced using carcinogen levels rarely encountered during normal exposures. The authors concluded that moderate dose (true exposure) and in-vivo (real exposure) situations should be kept in focus during the design as well as analysis of results from high dose bioassay and in-vitro experiments.

MODELING SYSTEM/TOXICOLOGICAL

83

Krutova TV, Chernikova SB, Konradov AA, Burlakova EB. THE EFFECT OF METHYLNITROSOUREA IN LOW DOSES ON THE STATIONARY CELL POPULATION: EXPERIMENT AND MODEL. Izv Ross Akad Nauk Ser Biol 1992; 0(4):511-518.

The change of stationary cell population (murine spleen) following an exposure of methylnitrosourea (10-11-10-5 g/kg of mouse weight) was investigated and modelled mathematically. The suggested model is based on the idea that the effect of cytotoxic agent in low doses has nondestructive "signal" character, changing dynamic balance between cells in proliferating and quiescent compartments.

84

Zucker RM, Elstein KH, Shuey DL, Kavlock RJ, Rogers JM. DEVELOPMENT OF BIOLOGICALLY BASED DOSE RESPONSE MODEL: FLOW CYTOMETRIC ANALYSIS OF THE MECHANISM OF ACTION OF 5-FLUOROURACIL (5-FU). Teratology 1992; 45(5):483.

Improving dose-response models for developmental toxicity requires an understanding of toxicant action at the cellular level. The have examined the cell cycle effects of 5-FU (an inhibitor of thymidylate synthetase) on several fetal tissues 8 and 24 hr after exposing GD14 rats to 5-40 mg/kg 5-FU. They observed that, in contrast to forelimb, hindlimb, palate, or torso, the DNA distribution of fetal liver nuclei 8 hr after exposure exhibits only minor S-phase acumulation. Moreover, 24 hr after exposure, liver nuclei exhibit apparent dose-dependent S-phase depletion. To understand this disparity in tissue response, we examined the time course of hepatic cell-cycle progression in utero and conducted in vitro synchronization and progression assays using a suspension-culture model. Findings indicate that within 4 hr of exposure to 5-FU, both types of cells accumulate in early S phase. Thereafter, affected cells progress synchronously through the cell cycle, resulting in either an apparent S-phase accumulation, S-phase depletion, or G2 block depending upon when cells were sampled. Examination of the DNA distribution of GD11-GD16 embryos revealed that cell cycle rate (as indicated by relative size of the S phase) varied with both embyronic age and tissue type, possibl by explaining the observed between-tissue variations. The observed consistent toxic action of 5-FU on the cell cycle of embryonic tissues is being related to the effects observed at term to develop a biologically based dose response model of 5-FU developmental toxicity.

85
Frederick CB, Potter DW, Chang-Mateu MI, Andersen ME.
A PHYSIOLOGICALLY BASED PHARMACOKINETIC AND PHARMACODYNAMIC MODEL TO DESCRIBE THE ORAL DOSING OF RATS WITH ETHYL ACRYLATE AND ITS IMPLICATIONS FOR RISK ASSESSMENT. Toxic Appl Pharm 1992; 114(2):246-260. (58 REFS)

A physiologically based pharmacokinetic model for the absorption, distribution, and metabolism of ethyl-acrylate in rats following oral dosing was developed. The major routes of metabolism were carboxylesterase catalyzed hydrolysis to acrylic-acid and ethanol and conjugation with glutathione, which was followed by binding to tissue proteins. Required biochemical constants were determined from in-vitro tissue metabolism studies. The model predicted that ethyl-acrylate would be very rapidly metabolized in all tissues. Attempts were made to validate the model by dosing male Fischer-F344-rats orally with 10, 50, or 100mg/kg ethyl-acrylate and determining the effect on tissue glutathione concentrations. In-vivo results followed predictions, and the authors conclude that the model

provides a means for understanding the response of rats to orally administered ethyl-acrylate. It also provides a quantitative basis for understanding target organ toxicity and the lack of toxicity in nontarget tissues that would be useful for risk assessments.

86
Auton TR. CONCENTRATION-FLUX RELATIONS FOR A MULTICELLULAR BIOLOGICAL MEMBRANE WITH METABOLISM. Math Biosci 1993;115(1):103-17.

A mathematical model is described for the simultaneous diffusion and metabolism of a chemical penetrating a multicellular biological membrane such as skin. Metabolism is assumed to follow saturable Michaelis-Menten kinetics, which leads to nonlinear relationships between the applied concentration and the metabolic and diffusive fluxes through the membrane. Approximate concentration-flux relations are derived under limiting conditions, and a computational method is described for the general case. The major barrier to dermal penetration of very lipophilic molecules is thought to be the viable tissues (viable epidermis and some of the dermis) underlying the stratum corneum, and some molecules are known to be metabolized by enzymes within these tissues. It is proposed to use the model to describe penetration and metabolism of such lipophilic molecules within the viable tissues of the skin.

87
Auton TR, Ramsey JD, Woollen BH. MODELLING DERMAL PHARMACOKINETICS USING IN VITRO DATA: PART I. FLUAZIFOP-BUTYL IN THE RAT. Hum Exp Toxicol 1993; 12(3):199-206.

The pharmacokinetics of the herbicide fluazifop-butyl have been determined in female rats following oral and intravenous dosing, and described by a mathematical model. Penetration of fluazifop-butyl through epidermal membranes has been determined using three different receptor fluids. It is demonstrated how this in vitro absorption data can be used with a pharmacokinetic model derived from oral and i.v. dosing studies to predict plasma concentrations and urinary excretion profiles following dermal dosing. Model predictions are compared with experimental measurements and found to be in good agreement.

MUTAGENESIS

88
Liu Y-Q, Robbins S, Zhong B-Z, Wallace WE, Jones W, Ong T. IN-VITRO MICRONUCLEUS ASSAY WITH KINETOCHORE ANALYSIS IN V79 CELLS TREATED WITH THREE GLASS FIBERS. 24th Annual Scientific Meeting of the Environmental Mutagen Society, Norfolk, Virginia, USA, April 17-22, 1993. Environ Mol Mutagen 1993; 21(SUPPL. 22):42.

No abstract.

89
Friend JH, Carpenter TD, Tice RR. EVALUATION OF CHEMICALLY-INDUCED DNA DAMAGE IN GERMINAL TISSUE OF FEMALE MICE USING THE SINGLE CELL GEL SCG ASSAY. 24th Annual Scientific Meeting of the Environmental Mutagen Society, Norfolk, Virginia, USA, April 17-22, 1993. Environ Mol Mutagen 1993; 21(SUPPL. 22):20.

No abstract.

90
Natarajan AT. AN OVERVIEW OF THE RESULTS OF TESTING OF KNOWN OR SUSPECTED ANEUGENS USING MAMMALIAN CELLS IN VITRO. Mutat Res;287(1):113-118.

Ten known or suspected aneugens were analyzed in a variety of in vitro mammalian cell cultures using different endpoints which included: micronuclei, kinetochore-positive micronuclei in binucleated cells, changes in the number of chromosomes or aberrations of mitosis and division. Human lymphocytes, human diploid fibroblasts and Chinese hamster transformed cells were used as target cells. The relative merits of different in vitro test systems employed are briefly discussed.

91
Lynch AM, Parry JM. THE CYTOCHALASIN-B MICRONUCLEUS/KINETOCHORE ASSAY IN VITRO: STUDIES WITH 10 SUSPECTED ANEUGENS. Mutat Res 1993; 287(1):71-86.

An in vitro micronucleus assay in low passage Chinese hamster Luc2 cells capable of detecting numerical and structural chromosome changes was developed. Chromosome loss was inferred by indirect visualisation of human CREST antikinetochore antibodies bound to centromeres in chemically-induced micronuclei of cytochalasin-B arrested binucleated cells. The assay was used to evaluate 10 chemicals which had been selected for their known or

suspected effects upon various components of the cell-division apparatus. These chemicals were colchicine (COL), vinblastine (VBL), thiabendazole (TBZ), chloral hydrate (CH), thimerosal (TM), diazepam (DZ), pyrimethamine (PYR), hydroquinone (HQ), cadmium chloride (CdCl2) and econazole nitrate (EZ). Mitomycin-C (MMC) was used as a positive control for the induction of micronuclei. The results with TM were equivocal and EN was negative. The results of these studies suggested that the cytochalasin-B Mn/k assay is a cost-effective, simple and rapid alternative to classical cytogenetic assays for the detection of chemically induced aneuploidy.

92
Warr TJ, Parry EM, Parry JM. A COMPARISON OF TWO IN VITRO MAMMALIAN CELL CYTOGENETIC ASSAYS FOR THE DETECTION OF MITOTIC ANEUPLOIDY USING 10 KNOWN OR SUSPECTED ANEUGENS. Mutat Res 1993;287(1):29-46.

Two in vitro cytogenetic assays were evaluated for their ability to detect aneugenic and polyploidy-inducing agents using a battery of 10 known or suspected aneugens supplied as part of the EEC 4th Environmental Research and Development Programme. The compounds tested were colchicine, vinblastine, chloral hydrate, thiabendazole, hydroquinone, thimerosal, cadmium chloride, econazole nitrate, pyrimethamine and diazepam. The cell division aberration assay employed a differential chromosome/spindle staining procedure to detect pertubations of the mitotic division apparatus. This assay was carried out in two pulmonary-derived Chinese hamster cell lines; the immortal DON: Wg3h culture and a low passage LUC2 culture. The second assay involved quantification of metaphase chromosomes, for which only the LUC2 cell line was used, due to the stability of its diploid karyotype. All the chemicals induced spindle disturbances in the immortal line. In addition, all the compounds except cadmium chloride yielded positive results in the LUC2 culture, although many were not as potent. The study of cell division aberrations was much less time-consuming and technically complex than the counting of metaphase chromosomes. In addition, it provided a degree of mechanistic understanding of the mode of action of some aneugenic and polyploidy-producing agents. However, the enumeration of chromosomes provides a more definitive data set for the evaluation of a chemical's aneugenic potential.

93
Hozier J, Applegate M, Moore MM. IN VITRO MAMMALIAN

MUTAGENESIS AS A MODEL FOR GENETIC LESIONS IN HUMAN CANCER.
Mutat Res 1992;270(2):201-209. (63 REFS)

The relevance of in-vitro mammalian mutagenesis assays as models for genetic alterations in human cancer is discussed. The mouse lymphoma assay (MLA) was used to detect a wide range of genetic lesions ranging from point mutations to chromosome rearrangements. This assay quantitated the potential of toxic chemicals to induce mutational inactivation of the autosomal thymidine-kinase gene. The MLA system permitted studies on the range of chemicals capable of inducing genetic lesions important to carcinogenesis, as well as on the nature of molecular events responsible for the lesions. The authors conclude that relevant in-vitro mammalian mutagenesis assays may be useful in distinguishing chemicals capable of inducing genetic lesions which develop into human cancer.

94
Kim JH, Thomas JJ. USE OF 4-(NITROBENZYL)PYRIDINE (4-NBP) TO TEST MUTAGENIC POTENTIAL OF SLOW-REACTING EPOXIDES, THEIR CORRESPONDING OLEFINS, AND OTHER ALKYLATING AGENTS. Bulletin of Environmental Contamination and Toxicology 1992;49(6):879-885. (22 REFS)

A chemical based test system for detecting the mutagenicity of alkylating agents and other genotoxic chemicals was developed. The system, known as the chemical activation system, was based on using ferrous ion and hydrogen-peroxide to simulate mammalian mixed oxidizing enzymes, and 4-(4-nitrobenzyl)pyridine (4-NBP) to determine the presence of alkylating agents. A mutagenic response was indicated by a significant increase in absorbance due to alkylation of the 4-NBP by the test agents. The authors conclude that the chemical activation system is a practical assay for elucidating the reactivity of indirect alkylating agents. Evidence of alkylation is assumed to be indicative of mutagenicity. The assay may prove to be a simple, nonbiological indicator of carcinogenic risk.

95
Bomhard EM, Bremmer JN, Herbold BA. REVIEW OF THE MUTAGENICITY/GENOTOXICITY OF BUTYLATED HYDROXYTOLUENE. Mutat Res 1992; 277(3):187-200. (89 REFS)

Various studies to determine the potential of butylated-hydroxytoluene (BHT) to cause point mutations were reviewed. In-vitro studies have been performed using various

bacterial species and strains and mammalian cell lines. In-vivo studies on Drosophila-melanogaster, silk worms, and the mouse specific locus test were also included. Findings from these studies did not indicate a potential for BHT to cause point mutations. The potential for BHT to cause chromosome aberrations was further investigated in in-vitro studies using plant cells and the WI-38, CHL, CHO, and V79 mammalian cell lines. Somatic and/or germ cells of D-melanogaster, rats, and mice were used for in-vivo studies. The lack of clastogenic potential on the part of BHT was demonstrated in nearly all of these investigations, particularly in those using validated test systems. No ability to damage or interact with DNA was shown through in-vitro studies on bacterial, yeast, and various mammalian cell lines including DON, CHO, CHL cells and primary hepatocytes. The authors concluded, based on the reviewed studies, that BHT does not represent a mutagenic or genotoxic risk to man.

96
Liu YX, Guttenplan JB. MUTATIONAL SPECIFICITIES OF N-NITROSAMINES IN A HOST-MEDIATED ASSAY: COMPARISON WITH DIRECT-ACTING N-NITROSO COMPOUNDS IN VITRO AND AN APPROACH TO DEDUCING THE NATURE OF ULTIMATE MUTAGENS IN VIVO. Mol Carcinog 1992; 6(4):232-7.

The mutational activities and specificities of several N-nitrosamines in Salmonella recovered from mouse liver in the host-mediated assay (HMA) were compared with the specificities of related direct-acting N-nitroso compounds in vitro. The specificities of the direct-acting Me, Et, Pr, and 2-hydroxypropyl compounds were all different and presumably are attributable to the DNA adducts resulting from the corresponding alkyldiazonium or carbonium ions. Introduction of a 2-hydroxyl group greatly influenced the mutational specificity. The 2-oxopropyl compound showed the same specificity as the Me compound. This result is consistent with one of the known breakdown pathways of the oxopropyl diazonium ion (or related reactive species), which leads to a Me diazonium ion. The N-nitrosodialkyl-nitro-sa-mines N-nitrosodimethylamine(NDMA), N-nitrosodiethylamine (NDEA), and N-nitrosodipropylamine (NDPA), which all require metabolic activation, showed specificities in the HMA similar to those of their direct-acting counterparts. The cyclic nitrosamine N-nitrosopyrrolidine was weakly active in the HMA, although its direct-acting derivative was a potent mutagen in vitro. The results for NDMA and NDEA were consistent with most previous studies of the metabolism of these compounds in vivo. The initial results described indicate that mutational specificity in the HMA can be used

to deduce metabolic pathways leading to genotoxic products
when the appropriate proximate mutagens are available as
standards. Furthermore, a reasonable correlation between
potency in the HMA and hepatocarcinogenesis was observed.

97
Gautam M, Nussear DL, Hong-Guang G, Clark NN.
MUTAGENICITY OF DIESEL EXHAUST PARTICULATE MATTER IN MINING
OPERATIONS. J Environ Sci Health Part A Environ Sci Eng
1993; 28(3):505-523.

Results from a study directed towards identifying and
measuring the mutagenicity of diesel exhaust particulate
matter involved in mining exposures, especially those which
may synergistically affect genotoxic hazard, are presented
in this paper. Particulate matter emissions from a diesel
engine, representative of the ones found in underground
mines, were sampled and assayed to determine the genotoxic
potential as a function of engine operating conditions.
Protocols developed for using a primary component of
pulmonary surfactant as a medium for collecting and applying
respirable particulate material to cells for mutagenicity
testing offer a true representation of in-vitro delivery of
such respired aerosols to pulmonary cells and the genetic
material within. Methods have been developed to sample and
assay the diesel soot and diesel soot/mineral dust complexes
which would not compromise the surface and physical
properties of the particulate material.

98
Clark LS, Nicklas JA, O'Neill JP, Jostes R, Albertini
RJ. IN-VITRO EFFECTS OF RADON EXPOSURE IN HUMAN G-O
T-LYMPHOCYTES USING THE HPRT CLONING ASSAY. 24th Annual
Scientific Meeting of the Environmental Mutagen Society,
Norfolk, VA, USA, April 17-22, 1993. Environ Mol Mutagen
1993; 21 (SUPPL. 22):14.

No abstract.

99
Jiao J, Glickman BW, Anderson MW, Zielinska M.
MUTATIONAL SPECIFICITY OF N-NITROSODIMETHYLAMINE: COMPARISON
BETWEEN IN VIVO AND IN VITRO ASSAYS. Mutat Res 1993;
301(1):27-31. (25 REFS)

Rat S9 activated N-nitrosodimethylamine (NDMA) was
treated with Aroclor-1254 and the resulting DNA alterations
were determined in the N-terminal region of the lacI gene of

Escherichia-coli. DNA sequencing was used to characterize a total of 125 independent LacI(-d) mutants of E-coli. The predominant mutation was the G/C to A/T transition. This transition accounted for about 90% of all the mutations recovered in this study. Other events observed included two G/C to T/A, two G/C to C/G, two A/T to G/C, three A/T to T/A and three frameshifts. After in-vitro activation with S9, contingency analysis was performed which revealed that the NDMA induced mutations had a spectrum which was similar to that obtained previously following in-vivo activation with a mouse host mediated assay. G/C to A/T events in each system significantly dominated the results and their distribution revealed similar site specificity. The proportion and kind of non G/C to A/T events were also similar.

100
Ficsor G, Goodwin JT, Leon JH, Rank K, Ginsberg LC. MUTATION DETECTION IN MAMMALIAN SPERM WITHOUT PROGENY TESTING AN IN-VITRO MODEL FOR METHOD DEVELOPMENT. 24th Annual Scientific Meeting of the Environmental Mutagen Society, Norfolk, VA, USA, April 17-22, 1993. Environ Mol Mutagen 1993; 21(SUPPL. 22):19.

No abstract.

NEPHROTOXICITY

101
McGuinness SA, Gandolfi AJ, Brendel K. USE OF RENAL SLICES AND RENAL TUBULE SUSPENSIONS FOR IN-VITRO TOXICITY STUDIES. In Vitro Toxicol 1993; 6(1):1-24.

No abstract.

102
Stary A, Menck CFM, Sarasin A. DESCRIPTION OF A NEW AMPLIFIABLE SHUTTLE VECTOR FOR MUTAGENESIS STUDIES IN HUMAN CELLS:APPLICATION TO N-METHYL-N'-NITRO-N-NITROSOGUANIDINE-INDUCED MUTATION SPECTRUM. Mutat Res 1992; 272(2):101-110. (37 REFS)

To analyze the mechanisms of mutagenesis in human cells, a 293-human-embryonic-kidney cell derived line containing a permanent mutagenesis target, the bacterial lacZ' gene, was established on an episomal EBV/SV40 based shuttle vector. Mutations produced in human host cells on the lacZ'locus were easily and rapidly scored and identified

in bacteria using the blue/white color assay. The lacZ' gene exhibited a low background frequency of point mutations over a 6 month period in culture. By treating cells with a potent mutagen, the efficiency of the system for detecting genotoxic induced mutations was investigated. The authors noted that the low spontaneous point mutation frequency on the mutagenesis locus and the ability to detect induced point mutations indicate that the system could be used in human mutagenesis studies at the molecular level.

103
Lan WG, Wong MK, Sin YM. IN VITRO EFFECT OF MERCURIC CHLORIDE ON ATPASE ACTIVITY IN KIDNEY OF THE FANCY CARP CYPRINUS CARPIO. Comp Biochem Physiol C Comp Pharmacol Toxicol 1993;104(2):307-310.

The Na+/K+-ATPase and Mg2+-ATPase activity of fancy carp (Cyprinus carpio) kidney homogenate without the presence of EDTA was significantly inhibited by mercuric chloride in a concentration-dependent manner. The Na+/K+-ATPase and Mg2+-ATPase activity of fancy carp (cyprinus carpio) kidney homogenate in the presence of EDTA showed no significant change with the increasing concentration of mercuric chloride. The sensitivity of Na+/K+-ATPase to mercuric chloride was greater than that of the Mg2+-ATPase. In conclusion, the use of EDTA in the determination of the effect of heavy metal on the ATPase activity as a toxicological index should be avoided. Using Na+/K+-ATPase activity as a toxicological index in the studies of heavy metal effect is better than using Mg2+-ATPase activity.

104
Charlton JA, Simmons NL. ESTABLISHED HUMAN RENAL CELL LINES: PHENOTYPIC CHARACTERISTICS DEFINE SUITABILITY FOR USE IN IN VITRO MODELS FOR PREDICTIVE TOXICOLOGY. Toxicol in Vitro 1993;7(2):129-36.

The phenotypes of the human renal epithelial cell lines, SK-NEP-1 and G401 (Wilm's tumor lines) and ACHN, A498, A704, Caki-1 and Caki-2 (renal adenocarcinomas), were investigated in order to develop toxicological model systems, human renal cell lines showing properties similar to those found in discrete renal tubular segments. All cell lines, except G401, demonstrated significant stimulation of adenyl cyclase activity by calcitonin. Alkakine phosphatase activity was not detectable in any cell line except for G401. None of the cell lines tested was capable of forming epithelial layers characteristic of tight epithelia. The

G401 cell line displayed several characteristics of the proximal nephron including a receptor for hPTH and detectable levels of the brush-border enzymes alkaline phosphatase (0.18 mU/mg protein), leucine aminopeptidase (14.0 mU/mg protein), glutathione transferase (8.61 mU/mg protein), and gamma-glutamyl transpeptidase (24.0 mU/mg protein). The effect of known nephrotoxic agents was tested on G401 cells by measuring basic mitochondrial enzyme function (MTT assay). The antibiotic gentamicin (5 mM)\ significantly inhibited MTT activity in a dose-dependent manner with a max. inhibition to 23.2% of untreated G401 cells. S-(1,1,2,2,Tetrafluoroethyl)-L-glutathione (4 mM) and its cysteine conjugate (2.5 mM) reduced MTT activity to 44.0% and 33.3% of control untreated G401 cells, respectively. It is suggested that the G401 cell line may be a useful model of the human proximal tubule in predictive toxicology.

105
Bach PH, Wilks MF. IN VITRO TECHNIQUES FOR NEPHROTOXICITY SCREENING, STUDYING MECHANISMS OF RENAL INJURY AND NOVEL DISCOVERIES ABOUT TOXIC NEPHROPATHIES IN IN VITRO METHODS TOXICOL 1992; [Proc. Int. Round Table Rhone-Poulenc Rorer Found.],6th;59-91. (200 REFS)

No abstract.

NEUROTOXICITY

106
Borowitz JL, Kanthasamy AG, Mitchell PJ, Isom GE. USE OF PC12 CELLS AS A NEUROTOXICOLOGICAL SCREEN: CHARACTERIZATION OF ANTICYANIDE COMPOUNDS. Fundam Appl Toxicol 1993;20(2):133-40.

A series of six biochemical markers of cyanide toxicity (dopamine release, hydroperoxide generation, cytosolic-free calcium levels, catalase activity, cytochrome oxidase activity, and superoxide dismutase activity) in cultured rat pheochromocytoma (PC12) cells were used to establish a screen for evaluation of potential anticyanide compounds. Thirty-nine substances, including anticonvulsants, adrenergic blockers, antioxidants, and antipsychotics were tested and ranked according to the results. Based on the composite scoring in all six assays, carbamazepine, mannitol, allopurinol, and phenytoin were ranked as the most effective anticyanide compounds. Additionally, known cyanide antidotes (e.g., pyruvate, mercaptopyruvate, alpha-ketoglutarate, naloxone, and flunarizine) obtained relatively high ranking in the PC12 cell screen. Also, a

significant correlation was found between protective effects (based on LD50s) of cyanide antidotes in mice and ranking in the in vitro screen. This study illustrates that by assaying a series of biochemical markers in a neuronal-type cell line, a rapid, cost-effective in vitro toxicological screen is possible. Several compounds have been identified which inhibit the biochemical effects of cyanide and may be used to enhance effectiveness of the standard cyanide antidotes.

107
Abdulla EM, Campbell IC. USE OF NEURITE OUTGROWTH AS AN IN VITRO METHOD OF ASSESSING NEUROTOXICITY. Ann N Y Acad Sci 1993;679:276-9.

This work shows that the neurotoxic excitatory amino acids beta-N-methylamino alanine, BMAA, and kainate, modulate neurite outgrowth; this was assessed by measuring the levels of two separate neurofilament proteins (68 kD and 160 kD), in a mouse neuroblastoma cell line, (NB41A3). BMAA has been proposed to be the exogenous excitotoxin in Guam disease or amyotrophic lateral sclerosis (ALS/parkinsonian/dementia; Guam ALS-PD). Kainate is a glutamate analogue which causes excitotoxic damage associated with excessive entry of calcium into neurons. The results show that at low doses (10(-9) to 10(-7) M) both BMAA and kainate decrease the concentration of the two neurofilament proteins. However at high doses (10(-6) to 10(-5) M) they cause an apparent accumulation of the neurofilament proteins; the effect is more marked with BMAA. These results support the continued development of an in vitro test for neurotoxicity based on neurite outgrowth.

108
Ali SF, LeBel CP, Bondy SC. REACTIVE OXYGEN SPECIES FORMATION AS A BIOMARKER OF METHYLMERCURY AND TRIMETHYLTIN NEUROTOXICITY. Neurotoxicology 1992; 13(3):637-648.
(41 REFS)

The formation of reactive oxygen species (ROS) as biomarkers for methylmercury (MeM) and trimethyltin (TMT) exposure was investigated. Adult C57B1/6N-mice received single doses of 1.0mg/kg MeM or 3.0mg/kg TMT, and CD-rats received single doses of 5.0mg/kg MeM. Mice were sacrificed 48 hours (hr) or one week (wk) after MeM treatment, and 24 and 48hr after TMT treatment. Rats were killed 1wk after MeM treatment. Synaptosomes were prepared from various regions of the brain, and assayed for ROS after incubation with nonfluorescent dichloro-fluorescein. Formation of the fluorescent product, dichlorofluorescein (DCF)

was monitored by spectrofluorometry. Control assays showed that DCF fluorescence was stimulated by ascorbate or ferrous-sulfate, while deferoxamine inhibited it. Results with the two organometals in-vitro showed that MeM increased the rate of formation of ROS, while TMT was without effect. In vivo, the rate of ROS formation in both rat and mouse cerebellum increased significantly after MeM treatment. The authors conclude that the increase in ROS formation in brain areas known to be specifically sensitive to organometals suggests that oxidative mechanisms may be responsible for the neurotoxic action of these compounds. DCF formation is a sensitive and direct measure of overall oxidative events, and can be used in both in-vitro and in-vivo situations.

109
Becking GC, Boyes WK, Damstra T, MacPhail RC. ASSESSING THE NEUROTOXIC POTENTIAL OF CHEMICALS. A MULTIDISCIPLINARY APPROACH. Environ Res 1993; 61(1):164-75.

Since 1981, the development of methodology to assess the neurotoxic potential of chemicals has been a high priority within the International Program on Chemical Safety (IPCS). Following the completion of an in-depth review of the scientific principles and methods for the assessment of neurotoxicity associated with chemical exposures, IPCS started to develop a multidisciplinary and comprehensive approach for neurotoxicity testing of chemicals. In view of the complexity of the nervous system and the variety of effects caused by chemicals, no single test or approach will be appropriate. Initially, IPCS evaluated neurobehavioral tests as well as in vitro procedures as screening tests, and an international collaborative study of neurobehavioral tests appropriate for screening chemicals for neurotoxicity is now in progress. Possible integration of higher level neurobehavioral tests with neurophysiological, biochemical, and other procedures in future testing strategies are discussed.

110
Abdulla EM, Campbell IC. IN VITRO TESTS OF NEUROTOXICITY. J Pharmacol Toxicol Methods 1993; 29(2):69-75. (31 REFS)

This review presents some of the newer techniques in the rapidly advancing area of neurotoxicity testing in vitro. They are not described at length, and more details can be obtained from the cited references. In vitro testing offers the possibility of relatively inexpensive screening of large numbers of pharmaceutical compounds, formulations,

and environmental substances. The level of sophistication attained in this field may soon allow much more accurate safety limits to be set, as specific mechanisms of neurotoxicity are elucidated.

111
Virmani MA, Dufour S, Corsico N, Catt KJ, Krsmanovic Z, Arrigoni-Martelli E. AN IN VITRO METHOD FOR EXAMINING THE EFFECTS OF PHARMACOLOGICALLY ACTIVE COMPOUNDS ON NEUROPEPTIDE SECRETION. Pharmacol Res 1993;27(Suppl 1):57-8.

The authors have developed an in vitro approach to enable the effects of various agents to be examined on the secretion of the neuropeptide, gonadotrophin releasing hormone (GnRH), from cultured hypothalamic neurons. Using this technique, they have demonstrated that the secretion of GnRH in vitro is dependent on the activity of voltage-dependent calcium channels. Also, acetyl-L-carnitine (L-Ac) improved the function of primary cultured rat fetal hypothalamic neurons by increasing GnRH secretion.

OCULAR TOXICITY
112
Vaughan JS, Porter DA. A NEW IN VITRO METHOD FOR ASSESSING THE POTENTIAL TOXICITY OF SOFT CONTACT LENS CARE SOLUTIONS. CLAO J 1993; 19(1):54-7.

The authors describe a method for determining the cytotoxic effect of soft contact lens care solutions. The method provides a means for the direct exposure of mammalian cells to solutions and to lenses that have been soaked in solutions. Cytotoxicity was indicated by significant increases in the number of dead cells relative to controls. The cytotoxicity of a test battery of eight compounds was determined, particularly benzalkonium chloride (BAK). In addition, nine commercial soft contact lens care products containing a variety of preserving/disinfecting agents were also examined. A modification of the basic assay for assessing the kinetics of toxicity is also described. The results indicate that this new method is useful for identifying compounds that bind to soft contact lenses, such as BAK, and induce cytotoxicity and others that should be rinsed from lenses or neutralized (such as hydrogen peroxide) to minimize the potential for irritation.

113
North-Root H, Yackovich F, Demetrulias J, Gacula M Jr, Heinze JE. EVALUATION OF AN IN VITRO CELL TOXICITY TEST

USING RABBIT CORNEAL CELLS TO PREDICT THE EYE IRRITATION POTENTIAL OF SURFACTANTS. Toxicol Lett 1982;14(3/4):207-212. (15 REFS)

An in-vitro toxicity test was developed using rabbit corneal cells to predict the eye irritation potential of cationic, anionic, nonionic, and amphoteric surfactant materials. New-Zealand-albino- rabbits were used for direct instillation of the surfactants into the eyes, and comparisons were made with cultured SIRC rabbit corneal cells incubated with surfactant. Different concentrations of test materials were added to the cultures, and were administered to the rabbits at a dose of 0.01 milliliter. Scoring was done at 24, 48, and 72 hours following applications using Draize scores. Two of the nonionic surfactants were relatively nonirritating. A rank correlation analysis showed that the order of eye irritancy, as well as cytotoxicity decreased from cationic to anionic/amphoteric to nonionic. The authors conclude that the in-vitro technique is a suitable screening method for testing product formulations for eye irritancy without use of live animals.

114
Calvin G. NEW APPROACHES TO THE ASSESSMENT OF EYE AND SKIN IRRITATION. Tox Let 1992; 64/65:157-164. (14 REFS)

New techniques for the evaluation of the eye and skin irritation potential of chemicals and consumer products were reviewed. Current regulations governing the assessment of eye and skin irritation potentials were discussed. The Low Volume Eye Test has been developed as an alternative to the traditional Draize eye test. Compared to the Draize test, the new method offers better correlation with actual human responses and is less stressful to the test animals. In-vitro irritation assays have been developed and the silicon microphysiometer, the luminescent bacteria toxicity test, and the neutral-red assay have yielded results considered promising for their use as alternatives to in-vivo testing. Other in-vivo tests being developed include alternative skin grading parameters using cutaneous blood flow measurement by Laser Doppler Flowmetry, infrared detection of skin temperature, and assessment of skin thickness. Open application cups, occlusive chambers, skin painting, and intradermal injection have been proposed as alternatives to the Draize occluded and semioccluded patch system. The use of the Human Skin Keratinocyte Cytotoxicity/Neutral-Red Assay for the in-vitro assessment of skin irritation potential was discussed.

115
Hurley PM, Chambers WA, Green S, Gupta KC, Hill RN,
Lambert LA, Lee CC, Lee JK, Liu PT, Lowther DK, et al.
SCREENING PROCEDURES FOR EYE IRRITATION. Food Chem Toxicol
1993;31(2):87-94. (28 REFS)

Screens aid in identifying some severe irritants or
corrosives and eliminating them from consideration for in
vivo eye irritation testing. Products may be evaluated for
ocular irritation potential in a stepwise progression as
follows: (1) products at pH extremes of 2 or below or of
11.5 or above may be considered to be ocular irritants; (2)
based on chemical structure-activity considerations, some
products may be judged to have ocular irritation potential;
(3) validated and accepted in vitro systems may possibly be
used as a screen in the future; (4) when a test material
demonstrates severe acute dermal toxicity (lethality at < or
= 200 mg/kg body weight), further testing for either dermal
or ocular irritation may not need to be undertaken;
(5) if a substance shows a primary dermal irritation
index of 5 or above, it may be considered to be an ocular
irritant; (6) materials that are not removed from
consideration based on these proposed screens may then be
considered for testing for ocular irritation in rabbits
under accepted procedures. Opinions on the use of the
individual screens varied between the different interested
groups attending the meeting, with the possoible future use
of in vitro screens for specific product lines having the
highest percentage of agreement (57-100%).

116
Green S, Chambers WA, Gupta KC, Hill RN, Hurley PM,
Lambert LA, Lee CC, Lee JK, Liu PT, Lowther DK, et al.
CRITERIA FOR IN VITRO ALTERNATIVES FOR THE EYE IRRITATION
TEST. Food Chem Toxicol 1993; 31(2):81-5. (18 REFS)

A proposal encompassing considerations and criteria for
the development of in vitro alternatives to the eye
irritation test has been developed and is presented in this
paper. Two factors need to be considered initially in
developing an alternative test. The first is to determine
whether the alternative assay is to be used as a screen or
as a replacement for the eye irritation test. Less stringent
acceptance criteria are required for an assay used as a
screen than for that used as a replacement test.
Screens answer fewer and less complex questions than a
replacement test would, since the results from screens
are usually confirmed by more definitive testing. A
replacement test, however, must provide the same answers as

in vivo methods for the assessment of eye irritation and must provide data for making a definitive toxicological assessment of eye irritation. The second factor to be considered is knowledge of the in vivo assay intended to be replaced. The following may be considered as criteria for in vitro tests used as screens or as replacements for the eye irritation test in rabbits: rationale (there should be a clear statement regarding the rationale for the use of a particular test in relation to the availability of other tests); relevance (the in vitro endpoint should have biological or physiological relevance to the effect to be detected in vivo); and validational (intralaboratory as well as interlaboratory validation must be conducted).

117
Gupta KC, Chambers WA, Green S, Hill RN, Hurley PM, Lambert LA, Liu PT, Lowther DK, Seabaugh VM, Springer JA, et al. AN EYE IRRITATION TEST PROTOCOL AND AN EVALUATION AND CLASSIFICATION SYSTEM. Food Chem Toxicol 1993;31(2):117-21.

An in vivo test protocol and an evaluation and classification system for the determination of eye irritation potential of chemicals and mixtures (substances) is proposed. The protocol uses two or three rabbits and reduces distress in test animals. The test substances are classified as non-irritant, irritant or severe irritant to meet regulatory needs. They may be classified on the basis of past experience with similar compounds or mixtures. Screens such as structure-activity relationships, pH extremes, validated and accepted in vitro tests, severe dermal irritation (primary dermal irritation index > or = 5) or severe dermal toxicity (lethality at < 200 mg/kg body weight) should be used to classify irritant or severe irritant materials when one or more of the screens can provide convincing evidence. For suspected severe irritant materials, the proposed in vivo test permits the use of one rabbit and instillation of 0.01 ml (0.01 g) of the test material into the cornea. Materials that are not classified irritant or severe irritant by screens or severe irritant by one rabbit test are tested in two or three rabbits. The material is classified as a severe irritant when the rabbit in the one-animal test or two or more rabbits in the standard test have responses of corneal opacity of 3 or above and iritis of 2 at 24, 48 or 72 hr, or positive responses on day 7 after instillation. The material is classified as an eye irritant when two or more rabbits are positive but the responses are not severe and they clear 7 days after instillation. The material is classified as a non-irritant when no more than one rabbit is positive.

118
Maertins T, Pauluhn J, Machemer L. ANALYSIS OF ALTERNATIVE METHODS FOR DETERMINING OCULAR IRRITATION. Food Chem Toxicol 1992; 30(12):1061-7.

An attempt was made to analyze the predictive power of the bovine eye-chicken egg chorioallantoic membrane (BE-CAM) assay in comparison with results obtained using the conventional Draize method. In summary, results showed limited correlation between reactions in vitro and responses of eyes in vivo. In a pilot study, ultrasonic pachymetry showed high sensitivity and fairly good correlation between corneal thickness and clin. observations in eyes.

ORGAN CULTURE

119
Beele H, Thierens H, Deveux R, Goethals E, De Ridder L. SKIN ORGAN CULTURE MODEL TO TEST THE TOXICITY OF POLYOXYETHYLENE NETWORKS. Biomaterials 1992; 13(14):1031-7.

Films of polyoxyethylene network were prepared from 2 types of triethoxysilane-terminated prepolymers. In this way, films of polyoxyethylene network with possible applications in the biomedical field could be made easier. To test their biocompatibility, these networks were added to organ cultures of adult human skin and embryonic chicken skin. A rapid toxic effect was obsd., esp. with the urethane-linked network. Enzymic degrdn. of the network by enzymes in the culture medium might be responsible for the formation of toxic metabolites. Testing of related chemical compounds in the in vitro assay suggested that the formation of a silane group with an amino terminal is most likely to be responsible for the toxic effects observed.

ORGAN TOXICITY

120
Comment CE, Blaylock BL, Germolec DR, Pollock PL, Kouchi Y, Brown HW, Rosenthal GJ, Luster MI. THYMOCYTE INJURY AFTER IN VITRO CHEMICAL EXPOSURE: POTENTIAL MECHANISMS FOR THYMIC ATROPHY. Journal of Pharmacology and Experimental Therapeutics 1992;262(3):1267-1273. (37 REFS)

The mechanisms by which certain chemicals may cause thymocyte injury and atrophy were investigated in-vitro. The thymuses of female F344-rats and B6C3F1-mice were removed and prepared in single cell suspensions. The compounds examined for direct toxicity on rodent thymocytes included

the following: acetaldehyde, acrolein, bleomycin-sulfate, cadmium-chloride (CdCl2), dexamethasone, dibenz(a,h)anthracene, dibutyltin-dichloride (DBTC), 2',3'-dideoxyadenosine, diethylstilbestrol, 3,4,5,3',4',5'-hexachlorobiphenyl,methoxyacetic-acid, nickel-sulfate, 2,3,7,8-tetrachlorodibenzo-p-dioxin, T-2-toxin, and 4-vinyl-1-cyclohexene-diepoxide. Changes in cell proliferation and viability were determined after an 18 hour culture period using 3 to 40 log molar concentrations of the test compounds. Acetaldehyde, acrolein, CdCl2, DBTC, and dexamethasone were the only compounds which affected thymocyte viability at in-vitro concentrations. The compounds that induced thymocyte atrophy also inhibited T-cell proliferation, but did not produce cell death. The authors conclude that the majority of compounds tested do not produce thymocyte death after direct in-vitro exposure, suggesting that indirect mechanisms may be involved in chemically induced thymic atrophy.

REPRODUCTIVE TOXICITY

121

Hamlin GP, Dukelow WR. TOXICITY OF EMULPHOR IN A MOUSE IN VITRO FERTILIZATION TEST SYSTEM. In Vitro Toxicology. J Mol Cell Toxic1992; 5(3):123-125. (3 REFS)

The reproductive toxicity of the surfactant Emulphor was examined in an in-vitro fertilization test system. Emulphor at concentrations of 0.001, 0.01, or 0.1% was incubated with oocytes and sperm from B6D2F1-mice in an organ tissue culture system for 29 hours. The cultures were then examined to determine the numbers of total ova and two cell embryos. The extent of fertilization was determined from the proportion of ova that developed into two cell embryos. The degree of fertilization in cultures exposed to 0.001, 0.01, and 0.1% Emulphor was 64.4, 49.2, and 23.0%, respectively. The control fertilization rate was 91.4%. The decreases in fertilization rate induced by Emulphor were statistically significant and linearly related to dose. The authors conclude that Emulphor is toxic to mouse gametes, causing a linear dose related decrease in fertilization rate.

122

Finnell RH, Mohl VK, Englen MD. IN VITRO ANALYSIS OF THE MURINE HEAT SHOCK RESPONSE: IMPLICATIONS FOR REPRODUCTIVE TOXICOLOGY. Toxicol Lett 1991; 58(3):297-308.

Lymphocytes isolated from two inbred mouse strains that differed in their genetically determined sensitivity to

heat-induced exencephaly were used to compare the in vitro kinetics of heat shock protein synthesis in the two strains following hyperthermic exposure. Differences in protein synthesis were determined by densitometric analysis of autoradiograms of SDS-PAGE gels. The findings were consistent with those observed in vivo in that there was an immediate and prolonged synthesis of heat-shock proteins by lymphocytes from the heat sensitive SWV/SD strain, compared to the response observed in lymphocytes from the heat-resistant DBA/2J strain. These results indicate that an in vitro lymphocyte assay of the heat-shock response may be a useful tool for screening suspected teratogenic agents.

123
Barber CV, Fantel AG. DNA STRAND DAMAGE BY ADRIAMYCIN AND NIRIDAZOLE: MICROGEL ELECTROPHORETIC ASSESSMENT OF INDIVIDUAL CELLS FROM CULTURED EMBRYOS. Teratology 1992; 45(5):499.

Measuring the genetic or nucleic acid damage induced by embryotoxicants is a central problem in reproductive toxicology. Alkaline elution and sedimentation are time-consuming and suffer some methodologic limitations. A microgel electrophoretic method was adapted for the quantitative analysis of DNA strand breaks in single cells. The system has the advantages of speed, sensitivity and the absence of radiolabels. In addition, large amounts of data may be generated from a few animals, with the potential for automation of data analysis and the ability to examine specific tissues, organs and cell types. We have examined the DNA-damaging effects of two drugs, adriamycin and niridazole, on GD-10 rat embryos during two hours of in vitro culture. Adriamycin produced a concentration-dependent, statistically significant increase in strand breaks over the embryotoxic range of concentration (0.5-4.0 uM). Niridazole induced no measurable DNA damage up to the totally embryolethal concentration (300 uM). Thus, while the toxicity of adriamycin may be mediated by DNA damage, the effects of niridazole appear to be produced by oxygen depletion through redox cycling.

124
Boadi WY, Urbach J, Brandes JM, Yannai S. IN VITRO EXPOSURE TO MERCURY AND CADMIUM ALTERS TERM HUMAN PLACENTAL MEMBRANE FLUIDITY. Toxicol Appl Pharmacol 1992; 116(1):17-23.
(50 REFS)

Potential alterations in placental membrane consistency

due to exposure to mercury (Hg) and cadmium (Cd) were investigated. An in-vitro system was used in which human placenta was incubated in medium with or without the metals. Determinations were made with reference to potential hazards to developing fetuses, and involved viability tests, an alkaline-phosphatase assay, steady state fluorescence polarization of placental membranes, phospholipid and cholesterol assays, metal analysis, and statistical evaluations. A 24 hour incubation with 6 or 12 micrograms per liter of either metal medium resulted in decreased fluidity with respect to control values. Observed changes were associated with effects on membrane function and damage to a developing fetus. The authors caution against extrapolation of animal study findings to human fetal effects due to morphological and physiological differences between the species.

125
Omarini D, Barzago MM, Aramayona J, Bortolotti A, Lucchini G, Bonati M. THEOPHYLLINE TRANSFER ACROSS HUMAN PLACENTAL COTYLEDON DURING IN VITRO DUAL PERFUSION. J Med 1992;23(2):101-16.

In vitro placental perfusion is widely used to investigate the placental transfer of endogenous compounds and, to a lesser extent, that of drugs. The aim of this study was to assess the suitability and reliability of such in vitro systems for application on drug placental transfer studies. The authors investigated the time course of theophylline (TH) transfer, a drug frequently used in the perinatal period. Eight experiments were performed with maternal and fetal circuits maintained in an open system, perfusing placentas for 160 min with Earle's enriched bicarbonate buffer containing two test substances, antipyrine (AP), (80 mg/L) and creatinine (CR), (150 mg/L), and the tool drug TH (15 mg/L). All substances equilibrated in our system with times proportional to the chemical-physical characteristics of each compound, being the time required to reach the steady state. Physiological conditions and biochemical properties of the tissue were well maintained throughout perfusions. Findings support the reliability of the technique to study transplacental passage of drugs, and the relevance of such a model to obtain information concerning potential therapeutic or toxicologic effects of drugs during the last trimester of pregnancy.

126
Anuszewska E. EFFECTS OF BUSULFAN ON EMBRYONIC CELLS IN VITRO. Pol J Pharmacol Pharm 1991; 43(2):129-33.

The DNA synthesis inhibition test and the DNA repair test have been used to study the effects of interaction between busulfan and DNA synthesis in two cell systems in vitro. The results of this study indicate that busulfan at concentration 500 and 1000 micrograms/ml damages mouse and human embryo cells. They also suggest that mouse embryo cells are unable to repair this damage.

127
Suzue T. A NOVEL METHOD FOR MAINTENANCE OF LIVE RODENT FETUSES IN LATE GESTATION IN VITRO AND ITS APPLICATION FOR DEVELOPMENTAL STUDIES. Teratology 1991; 44(6):6B-7B.

A new method was developed for maintaining physiological activities of late gestation fetuses of the rat and mouse in vitro (T. Suzue, Proceedings of the Japan Academy, Vol 66 (1990)177-181.). Fetuses that are functionally connected with uterus through placenta and umbilical cord were isolated from pregnant rats (E14-E20) and were perfused through a uterine artery with a physiological salt solution containing general anaesthetics. After decerebration, preparations were perfused with a physiological solution without general anaesthetics. Under this condition, fetuses showed heartbeats and spontaneous body movements. It was possible to evoke body movements by electrical stimulation of the skin. Both the spontaneous and stimulation-induced

body movements were depressed after perfusion with general anaesthetics i.e., ether, halothane or pentobarbital, suggesting that the movements are controlled by the activities of the central neurons. The fact that the activities of the central nervous system as well as that of the heart were retained under present conditions implies that the general condition of the fetuses is good. Therefore, the present method may be useful for studying the mechanism of normal and abnormal development of mammalian fetuses.

RESPIRATORY TOXICOLOGY

128
Wallaert B, Voisin C. IN VITRO STUDY OF GAS EFFECTS ON ALVEOLAR MACROPHAGES. Cell Biology and Toxicology 1992; 8(3):151-156. (22 REFS)

An aerobic culture system for examining the effects of toxic gases on alveolar macrophages was described. Alveolar macrophages harvested by bronchoalveolar lavage were layered on a Gelman membrane which was placed on the surface of a

reservoir filled with culture medium. The cells were maintained in air containing 5% carbon-dioxide at 37 degrees-C. The culture system was tested by exposing the cells to 0, 0.2, 2, or 5 parts per million (ppm) nitrogen-dioxide in air containing 5% carbon-dioxide for 30 minutes or 24 hours. Cytotoxicity was assessed by determining changes in adenosine-triphosphate (ATP) content or beta-glucuronidase activity or by observing the cells for morphological changes. The effects on superoxide production and chemotactic activity on neutrophils were also examined. Twenty four hours exposure to 0.2ppm nitrogen-dioxide caused a significant decrease in ATP content. Twenty four hours exposure to 2 and 5ppm nitrogen-dioxide completely destroyed the cells. The authors conclude that the cell culture system allows direct contact between the test gas and the alveolar macrophages. This eliminates the barrier presented by the culture medium and reproduces the physiological conditions of the bronchoalveolar spaces. The study shows that gaseous air pollutants can injure or activate alveolar macrophages.

129
Kondo T, Takahashi S, Sato H, Yamada M, Kikuchi T, Furuya K. CYTOTOXICITY OF SIZE-DENSITY FRACTIONATED COAL FLY ASH IN RAT ALVEOLAR MACROPHAGES CULTURED IN VITRO. Toxicol In Vitro 1993;7(1):61-67.

The cytotoxicity of coal fly ash particles was investigated in rat alveolar macrophages cultured in vitro. The exclusion of trypan blue and the extracellular release of lactate dehydrogenase were used as indicators of cell viability. The fraction of fly ash particles with the highest density (>2.8 g/m3) was most cytotoxic. The degree of cytotoxicity appeared to be correlated with the amount of particles taken up by macrophages as well as with their heavy metal concentrations. Morphological observations of the surface of the macrophages by scanning electron microscopy revealed a decrease in the number of filopodia and microvilli and an enhancement of the ruffled membrane. The morphological changes became more obvious with increasing concentrations of fly ash particles, but there was no characteristic change specific to each fraction of fly ash.

130
Romet-Haddad S, Marano F, Blanquart C, Baeza-Squiban A. TRACHEAL EPITHELIUM IN CULTURE: A MODEL FOR TOXICITY TESTING OF INHALED MOLECULES. Cell Biology and Toxicology 1992; 8(3):141-150. (20 REFS)

A tracheal mucociliary epithelial cell assay for assessing

the specific toxicity of air pollutants was developed. Mucociliary epithelium outgrowths prepared from Fauve-de-Bourgogne-rabbit tracheal explants were attached to collagen gel coated filters that were inserted in Millicell culture chambers. The general acute toxicity of the test agent was assessed by determining viability using the MTT assay and by measuring neutral-red-dye uptake and release of lactate-dehydrogenase (LDH) into the culture medium. The effects of the test agent on culture growth were evaluated by a microcomputer controlled image analysis system that measured the area covered by the explant and outgrowth. Ciliotoxicity was assessed by measuring ciliary beat frequency of individual ciliated cells using a digitized image analysis system. The effects of the test agent on transepithelial potential were determined using a silver/silver-chloride electrode and agar bridge system. The assay was tested using mechlorethamin and acrolein. The authors conclude that the assay offers an approach to assess the mechanisms of toxicity of inhaled chemicals as well as their acute cytotoxicity.

131
Becher R, Lag M, Schwarze PE, Brunborg G, Soderlund EJ, Holme JA. CHEMICALLY INDUCED DNA DAMAGE IN ISOLATED RABBIT LUNG CELLS. Mutat Res 1993; 285(2):303-311. (59 REFS)

Induction of DNA damage by methylmethane-sulfonate (MMS), 1,2-dibromo-3-chloropropane (DBCP), 1-nitropyrene (1NP), 2-nitro-fluorene (2NF), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N'-nitrosonornicotine (NNN), N-nitrosoheptamethyleneimine (NHMI), and phorbol-12-myristate-acetate (TPA) was studied in isolated rabbit lung cells. Clara and type-II cells and alveolar macrophages were lavaged from the lungs of New-Zealand-White-rabbits. The cells were then incubated with 0 to 3000 micromolar (microM) of the chemical. The extent of DNA damage was determined by an alkaline elution assay. MMS induced a dose dependent increase in DNA damage that was similar in all three cell types. DBCP at 30microM caused significant damage in Clara and type-II cells; a concentration of 300microM was necessary to induce the same level of damage in alveolar macrophages. NNK at 30microM caused significant DNA damage in Clara and type-II cells. Significant damage to alveolar macrophages was caused only by the 1000microM dose. NHMI at 300microM induced significant DNA damage in Clara and type-II cells; no DNA damage was induced in alveolar macrophages. TPA at 15microM induced DNA damage in Clara cells. A concentration of 50microM was required to cause damage in type-II cells. TPA did not induce DNA damage in alveolar macrophages. NNN, 1NP, and 2NF did not induce DNA

damage in any of the cell types. The authors conclude that isolated lung cells combined with alkaline elution can be used to study chemically induced pulmonary cell DNA damage.

132
Brain JD. SHORT-TERM IN VITRO AND IN VIVO BIOASSAYS: THEIR ROLE IN ESTIMATING THE TOXIC POTENTIAL OF INHALED COMPLEX MIXTURES FOR HUMANS. Cell Biol Toxic 1992; 8(3):123-132. (30 REFS)

Assessing the toxic potential of complex particulate mixtures was discussed. Little or no data was available for risk assessment of volcanic ash from Mount Pinatubo in the Philippines, soot from oil wells in Kuwait set afire as a result of the Persian Gulf War, continued exposure to particulates in urban air, and increased exposure to indoor air pollutants associated with closed building syndrome. Risk assessment techniques such as air monitoring and physicochemical characterization of collected aerosols, epidemiological studies, controlled experimental human studies, lifetime experimental animal studies, short term animal bioassays, and in-vitro tests were discussed. The discussion also involved a bioassay developed by the author which was based on assessing cellular and biochemical changes induced in bronchoalveolar lavage fluid of hamsters and rats by inhaled complex mixtures. The animals were exposed to the mixtures by inhalation or intratracheal instillation. The bioassay was calibrated by exposing the animals to dusts for which considerable human data exist. Overall, new techniques for studying pollutant effects on alveolar macrophages were described.

133
Knorst MM, Kienast K, Muller-Quernheim J, Ferlinz R. [ALVEOLAR MACROPHAGES AND MONONUCLEAR CELLS OF THE PERIPHERAL BLOOD IN SULFUR DIOXIDE AND CHRYSOTILE B EXPOSURE: A REALISTIC IN VITRO TEST OF OXYGEN FREE RADICAL LIBERATION]. Pneumologie 1993;47(5):353-6.

Sulfur dioxide (SO2) and Asbestos are frequently found at workplaces. They can induce airway and lung parenchymal injury. Alveolar macrophages (AM) play an important and decisive role in the damage of respiratory tissue. We evaluated the reactive oxygen intermediates (ROI) production of AM and peripheral blood mononuclear cells after exposure to SO2 and Chrysotile B. The cells were exposed in a special gas exposure chamber at 37 degrees C and 100% air humidity for 30 minutes to 1.5 or 2.5 ppm SO2. Afterwards they were incubated for one hour with 100 micrograms or 200 micrograms

Chrysotile B. Control experiments were performed with cell exposure to synthetic air without SO2 and Chrysotile B. Spontaneous and phorbol myristate acetate (PMA) stimulated ROI-release were measured by chemiluminescence and the cell toxicity was evaluated with the trypan blue exclusion test. The results show a dose-dependent increase of the spontaneous ROI-production of AM after SO2 and Chrysotile B exposure.

134
Lewtas J, Mumford J, Everson RB, Hulka B, Wilcosky T, Kozumbo W, Thompson C, George M, Dobias L, Sram R, et al. COMPARISON OF DNA ADDUCTS FROM EXPOSURE TO COMPLEX MIXTURES IN VARIOUS HUMAN TISSUES AND EXPERIMENTAL SYSTEMS. Environ Health Perspect 1993;99:89-97.

DNA adducts derived from complex mixtures of polycyclic aromatic compounds emitted from tobacco smoke are compared to industrial pollution sources (e.g., coke ovens and aluminum smelters), smoky coal burning, and urban air pollution. Exposures to coke oven emissions and smoky coal, both potent rodent skin tumor initiators and lung carcinogens in humans, result in high levels of DNA adducts compared to tobacco smoke in the in vitro calf thymus DNA model system, in cultured lymphocytes, and in the mouse skin assay. Using tobacco smoke as a model in human studies, the authors have compared relative DNA adduct levels detected in blood lymphocytes, placental tissue, bronchoalveolar lung lavage cells, sperm, and autopsy tissues of smokers and nonsmokers. Comparison of the DNA adduct levels resulting from human exposure to different complex mixtures shows that emissions from coke ovens, aluminum smelters, and smoky coal result in higher DNA adduct levels than tobacco smoke exposure. The studies suggest that humans exposed to complex combustion mixtures will have higher DNA adduct levels in target cells (e.g., lung) as compared to nontarget cells (e.g., lymphocytes) and that the adduct levels will be dependent on the genotoxic and DNA adduct-forming potency of the mixture.

135
Gallagher J, George M, Kohan M, Thompson C, Shank T, Lewtas J. DETECTION AND COMPARISON OF DNA ADDUCTS AFTER IN VITRO AND IN VIVO DIESEL EMISSION EXPOSURES. Environ Health Perspect 1993;99:225-8.

Development of methods to evaluate certain classes of polycyclic aromatic compounds (PAC) detected in complex mixtures to which humans are exposed would greatly improve

the diagnostic potential of 32P-postlabeling analysis. Identification of DNA adduct patterns or specific exposure-related marker adducts would strengthen associations between observed DNA adducts and exposures to different environmental pollutants (e.g., kerosene, cigarette smoke, coke oven, and diesel). We have compared diesel-modified DNA adduct patterns in various in vitro and in vivo rodent model systems and compared them to DNA reactive oxidative and reductive metabolites of nitropyrene. The formation of nitrated polycyclic aromatic hydrocarbon (nitrated PAH) DNA adducts, derived from the metabolism of diesel extract constituents, was enhanced relative to other PAH-derived DNA adducts via xanthine oxidase-catalyzed nitroreduction. These adducts were detectable only by the butanol extraction version of the postlabeling analysis. A major adduct detected in human lymphocytes treated in vitro with diesel extract comigrated with a major adduct detected in lymphocyte DNA treated with benzo[a]pyrene (BaP) alone. Postlabeling of lung DNA isolated from rodents exposed via lung inhalation for 24 months to diesel combustion emissions resulted in the formation of a major nuclease-P1-sensitive DNA adduct that did not co-migrate with the major BaP-diol epoxide adduct.

136
Dolan LR, Rutberg SE, Emura M, Mohr U, Hoffmann D,Hecht SS, Ronai Z. IN-VITRO TRANSFORMATION ASSAY OF CLARA CELLS A POSSIBLE MODEL FOR THE STUDY OF LUNG CARCINOGENS. 84th Annual Meeting of the American Association for Cancer Research, Orlando, Florida, USA, May 19-22, 1993. Proc Am Assoc Cancer Res Annu Meet 1993;34(0):174.

No abstract.

137
Hazinski TA. GENE TRANSFECTION OF LUNG CELLS IN VITRO AND IN VIVO. Annu Rev Physiol 1993; 55:181-207. (151 REFS)

No abstract.

SKIN TOXICITY

138
Jain PT, Fitzpatrick MJ, Phelps PC, Berezesky IK, Trump BF. STUDIES OF SKIN TOXICITY IN VITRO: DOSE-RESPONSE STUDIES ON JB6 CELLS. Toxicol Pathol 1992; 20(3):394-404.

There are many reasons for developing in vitro tests of toxicity including cost, speed, studies of mechanisms, and

studies utilizing human cells and tissues. The present study focuses on the development of in vitro tests to predict in vivo toxicity by comparing them to data from the literature. A broad spectrum of model toxic compounds was evaluated for toxicity on mouse skin JB6 cells in culture. These included mercuric chloride, sodium lauryl sulfate, formaldehyde, dimethyl sulfoxide, benzoyl peroxide, and ionomycin, all of which have been proven to be positive in the Draize test or in cutaneous toxicity studies. Cell viability was evaluated every 15 min for up to 1 hr, and then after 24 hr of treatment using the Trypan Blue exclusion method; morphological changes were evaluated using phase-contrast and transmission electron microscopy. Dose- and time-dependent cell death and morphological changes were observed at concentrations ranging from 10(-14) to 10(-2) M. The findings suggest that the JB6 cell culture model can be used for predicting in vivo toxicity. In the future, it may be possible to utilize this system for the study of intracellular ionized calcium ([Ca2+]i), and the expression of oncogenes as early indicators of toxicity.

139

Barber ED, Teetsel NM, Kolberg KF, Guest D. A COMPARATIVE STUDY OF THE RATES OF IN VITRO PERCUTANEOUS ABSORPTION OF EIGHT CHEMICALS USING RAT AND HUMAN SKIN. Fund Appl Toxicol 1992;19(4):493-497. (27 REFS)

The comparative rates of absorption of water, 2-ethoxyethyl-acetate (EEA), diethylene-glycol-monobutyl-ether (DGBE), urea, di(2-ethylhexyl)phthalate (DEHP), 2-ethylhexanol (2EH), ethyl-3-ethoxypropionate (EEP) 2-propoxyethanol (2PE) through human and rat skin were studied in-vitro. Tritiated water or carbon-14 labeled EEA, DGBE, urea, DEHP, 2EH, EEP, or 2PE was applied to full thickness skin from male Sprague-Dawley-rats and human stratum corneum mounted on Franz type diffusion cells. The preparations were incubated in the diffusion cells for 8 hours except in the case of DEHP and 2EH in which the incubation period was 32 hours. Build up of radioactivity in the receptor compartment and decrease of radioactivity in the donor compartment were determined for each compound. The absorption rates of the test compounds in milligrams per square centimeter per hour through full thickness rat skin were determined. The authors conclude that rat skin is more permeable to the test chemicals than human skin. The data indicate that using in-vitro rat skin data may overestimate the degree of skin absorption in humans.

140

Klain GJ, Reifenrath WG. IN VIVO ASSESSMENT OF DERMAL ABSORPTION. Dermal and Ocular Toxicology: Fundamentals and Methods 1991, D. W. Hobson, Editor; CRC Press, Boca Raton, Florida, pages 247-266. (63 REFS)

Experimental procedures for investigating in-vivo dermal absorption of chemical compounds were reviewed. The basic principles of and problems associated with performing in-vivo dermal absorption research were considered. Laboratory animals are used extensively in dermal absorption studies since many of the studies cannot be performed in humans. The results are used to predict percutaneous absorption in humans. Marked differences exist in the dermal absorption of chemicals between humans and animals; this complicates extrapolation of results to humans and duplication of dermal absorption characteristics of a large number of chemicals in humans in an animal model. The methodology for performing in-vivo dermal absorption studies was reviewed. Indirect methods were based on determining the amounts of test compounds (usually radiolabeled) that were excreted in the feces or urine. Indirect methods can be used with human subjects if the test compound is not too toxic; direct methods cannot be used in humans. Consideration was also given to presenting and analyzing data obtained in in-vivo dermal absorption studies and comparing the results of in-vitro and in-vivo dermal absorption measurements.

141
Collier SW, Storm JE, Bronaugh RL. REDUCTION OF AZO DYES DURING IN VITRO PERCUTANEOUS ABSORPTION. Toxicol Appl Pharmacol;118(1):73-9.

The azo reductions of Sudan I, aniline subsidiary color of FD&C Yellow No. 6 (ANSC)], and Solvent Yellow 7 (SY7) in skin during percutaneous absorption was measured and the contributions of cytosolic and microsomal reductions were characterized. By using a series of azo dyes with a common U-14C-labeled phenylazo moiety, percutaneous absorption and metabolism were measured in vitro inflow-through diffusion cells with Sencar mouse, hairless guinea pig, and human skin. Azoreductase assays using subcellular fractions from skin of all species were used to examine intracellular rates and distribution for the series of dyes. The absorption of the lipophilic dyes Sudan I and SY7 from a finite surface dose of 5 mug/cm2 was extensive in all species. Human skin was least permeable, with 26.4% of the applied Sudan I dose and 36.1% of the applied SY7 dose absorbed in 24 h. Sudan I and SY7 were extensively reduced in skin of all species during precutaneous absorption (29.5 and 26.5%,

respectively, of the absorbed dose in human skin and greater than 50% of the applied dose in other species). The extensive azo reduction observed during percutaneous absorption may modulate the toxicities of these compounds and must be considered when EDs are determined for quantitative risk assessments from dermal exposures.

142
Hadgraft J, Beutner D, Wolff HM. IN VIVO-IN VITRO COMPARISONS IN THE TRANSDERMAL DELIVERY OF NITROGLYCERIN. Int J Pharm 1993;89(J):R1-R4. (4 REFS)

Reported correlations between the in vitro release of transdermal patch formulations of nitroglycerin, Deponit, and Nitro-Dur II, across human skin dermatomed to 220 mum. Plasma levels in vivo are discussed. It was noted that there was very good agreement between predicted and experimentally determined values for both preparations. This showed that the in vitro method linked with clearance kinetics can provide information about the delivery of the drug over the time course of application. It was concluded that, for the delivery of transdermal nitroglycerin, the method in which drug release was assessed with the patch in contact with human skin dermatomed to 220 mum, there appears to be very good in vitro-in vivo correlation.

143
Green P, Shroot B, Bernerd F, Pilgrim WR, Guy RH. IN VITRO AND IN VIVO IONTOPHORESIS OF A TRIPEPTIDE ACROSS NUDE RAT SKIN. J Controlled Release 1992; 20(Aug):209-217. (19 REFS)

To examine the predictability of in vitro iontophoretic measurements for the in vivo delivery of a positively charged tripeptide, threonine-lysine-proline (threonyl-lysyl-proline), the iontophoretic delivery across nude rat skin of the peptide in a poloxamer 407 gel was measured both in vivo and in vitro. Transport of the tripeptide across the skin was proportional to the applied continuous direct current density. Minimal degradation by electrolysis and/or metabolism of the peptide was observed over a 6 hr. study period. Iontophoretic pretreatment of the skin at the peptide application site decreased barrier function. Similar results were obtained following iontophoretic delivery of the tripeptide both in vivo and in vitro. It was concluded that iontophoresis is an effective, yet relatively inefficient, method to enhance delivery of a positively charged tripeptide into and through the skin.

144
Kao J. IN VITRO ASSESSMENT OF DERMAL ABSORPTION in Dermal and Ocular Toxicology: Fundamentals and Methods, D. W. Hobson, Editor; CRC Press, Boca Raton, Florida, pages 267-295, 144 references, 1991.

Procedures for investigating in-vitro dermal absorption of chemicals were reviewed. The nature of and reasons for studying dermal absorption were considered. Studies of dermal absorption have become an important component of dermatotoxicological research. Increasing interest has also been expressed regarding transdermal exposure as a means of delivering drugs for systemic therapy. The general principles of in-vitro skin absorption methodology were reviewed. In-vitro dermal absorption studies are based on the assumption that the skin is a diffusional membrane and that the problem of skin absorption can be described quantitatively by diffusion equations and mass transfer coefficients. In-vitro skin absorption

investigations are usually conducted using diffusion chambers in which the skin preparation serves as the diffusion membrane. The diffusion chambers are usually assembled with the skin preparation separating the donor and receptor compartments. An appropriate fluid is placed in the receptor compartment and the test chemical (usually radiolabeled) is placed in the donor compartment. The degree of penetration of the test compound through the skin preparation is estimated fro the amount of radiolabel recovered from the receptor compartment. Problems to consider when performing in-vitro dermal absorption studies included: establishing and maintaining the integrity of the skin preparation, maintaining the metabolic viability of the preparation, the toxicity of the test compound, selecting appropriate environmental conditions, and recovering the test compound.

145
Michniak BB, Seyda KL. A SIMPLE AND SPECIFIC HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE ASSAY OF A SERIES OF NOVEL DERMAL PENETRATION ENHANCERS. Int J Cosmet Sci 1993;15(1):7-13.

A series of clofibric acid amides was synthesized and previously reported by the authors as possessing enhancer activity in vitro in athymic nude mouse skin against model drugs, hydrocortisone 21-acetate and beta-methasone 17-valerate. An assay was required for each of these enhancers however, which would be specific for each compound

and would also separate model drugs and their metabolite peaks. This study reports reversed-phase HPLC assays for clofibric acid amide and 7 derivatives. All enhancers showed maximum absorption at 232 nm, betamethasone (BM) and its valerate (BMV) at 238 nm, and hydrocortisone (HC) and its acetate (HCA) at 242 nm. Practical units of detection for the amides were 0.46-2.8 mug-mL-1 and peaks were sharp and well separated from steroid peaks in 3 vehicles, methanol alone, Franz diffusion cell receptor phase samples (isotonic phosphate buffer), and full-thickness athymic nude mouse skin extracts in methanol. Mobile phases consisted of various proportions of acetonitrile and water, some with 2-propanol. The octyl amide for example, with mobile phase of CH3CN-H2O (85:15) at 1 mL min-1 had a retention time (tR) of 7.9 minutes. Under the same conditions, retention times for the steroids were 3.3 min for HC, 4.3 min for HCA, 3.4 min for BM, and 4.6 min for BMV.

146
Michniak BB, Player MR, Chapman JM Jr, Sowell JW. IN VITRO EVALUATION OF A SERIES OF AZONE ANALOGS AS DERMAL PENETRATION ENHANCERS. Int J Pharm 1993; 91(1):85-93.

The influence of a series of Azone analogs on the percutaneous penetration of a lipophilic model drug (hydrocortisone 21-acetate) across hairless mouse skin has been investigated. Methods of synthesis of these novel compounds are also described. Permeability studies utilized vertical non-occluded Franz cells at 37.degree. and propylene glycol as the vehicle for the drug. Enhancers were applied one h prior to drug treatment in the same vehicle. Three enhancers were applied at their maximum saturation solubilities in propylene glycol, the rest of the compounds at 0.4 M. Enhancement ratios were calculated for flux, 24 h diffusion cell receptor concentrations, and full-thickness skin total steroid contents. All enhancers were found to increase permeation parameters to a greater or lesser extent over control. A few compounds were found to be more effective than Azone in increasing these parameters; particularly skin retention of the model drug.

STRUCTURE/ACTIVITY TOXICITY
147
Kavlock RJ, Oglesby LA, Hall LL, Fisher HL, Copeland F, Logsdon T, Ebron-McCoy M. IN VIVO AND IN VITRO STRUCTURE-DOSIMETRY-ACTIVITY RELATIONSHIPS OF SUBSTITUTED PHENOLS IN DEVELOPMENTAL TOXICITY ASSAYS. Reprod Toxicol 1991;53:255-8.

Structure-dosimetry-activity relationships (SDARs) of a series of substituted phenols were evaluated following exposure of gestation day 11 rats in vivo and in comparable stage embryos in vitro. In the in vivo study, 27 congeners were assayed and log P (a term used synomously with lipophilicity in this paper) and Hammett sigma values (a measure of the electronic withdrawing ability of the substituent) were shown to correlate with maternal toxicity; however, no relationships between these parameters and developmental effects were observed. In the in vitro system, 13 congeners were evaluated and molar refractivity and/or lipophilicity were shown to correlate with the ability of the phenols to induce embryonic growth retardation and structural defects in the absence of the hepatocytes. In contrast, when a metabolic activating system (primary hepatocytes) was present in the in vitro system, the potential to induce growth retardation was inversely related to lipophilicity. Correcting the in vitro potency data for the variable amount of binding to macromolecules improved the predictiveness of the quantitative structure-activity relationships (QSARs). The potential to induce embryotoxicity in vitro was not well correlated with the potential to induce developmental toxicity in vivo: whereas the in vitro data demonstrates that the phenols are intrinsically embryotoxic, few of them actually produced significant developmental toxicity in the in vivo system, and there were few positive correlations between effects observed in the two systems.

148
Naish-Byfield S, Cooksey CJ, Latter AM, Johnson CI, Riley PA. IN VITRO ASSESSMENT OF THE STRUCTURE-ACTIVITY RELATIONSHIP OF TYROSINASE-DEPENDENT CYTOTOXICITY OF A SERIES OF SUBSTITUTED PHENOLS. Melanoma Res 1991; 1(4):273-87.

The rate of oxidation of 30 compounds by purified mushroom tyrosinase was measured by using oximetry; the tyrosinase-dependent cytotoxicity of each compound was established by an in vitro assay using exposure of nonmelanogenic cells to the agents in the presence and absence of tyrosinase. Cytotoxicity was established by immediate inhibition of DNA synthesis; 4-hydroxyanisole was used as the reference material. Compounds that were not oxidized by tyrosinase were found to be nontoxic but there was no direct relationship between the rate of oxidation and the relative cytotoxicity of those materials that acted as substrates for the enzyme.

149
Fisher HL, Sumler MR, Shrivastava SP, Edwards BC,
Oglesby LA, Ebron-McCoy MT, Copeland F, Kavlock RJ, Hall LL.
IN VITRO PHARMACOKINETICS AND STRUCTURE-TOXICITY OF
SUBSTITUTED PHENOLS IN DEVELOPMENTAL TOXICITY ASSAY.
Toxicologist 1992;12(1):162.

Uptake of 9 14C para-substituted phenols by day 10 rat
embryos in vitro was determined from 1 to 42 hr after being
placed in dosed culture media. Uptake was rapid, with 7 of 9
compounds having an uptake half-time of less than 1 hr.
Equilibrium concentration in embryos ranged from 53 to 135%
of the media concentration indicating only a factor of 2 in
maximum discrimination of uptake for any phenol studied.
When hepatocytes were also present the equilibrium
concentration in the embryo was less than that observed when
hepatocytes were not present. For 4 phenols there was no
(i.e. 0) new equilibrium level. Thus the metabolites
produced by hepatocytes appear to have less affinity for the
embryo than the parent phenol. Binding of phenols by
media constituents increased as log p (octanol/water
partition coefficient) increased. The effective dose for
producing tail defects and reduction in somite number for 42
hr exposure was about constant for phenols with log p less
greater than 1.9. The effective dose was larger (reduced
toxicity) for phenols with log p less than 1.9. Chemical
structure of substituted phenols affects uptake, binding,
and teratological abnormalities in rat embryos in vitro.

150
Stijntjes GJ, Commandeur J NM, Te Koppele JM, McGuinness S,
Gandolfi AJ, Vermeulen N PE. EXAMINATION OF THE
STRUCTURE-TOXICITY RELATIONSHIPS OF L-CYSTEINE-S-CONJUGATES
OF HALOGENATED ALKENES AND THEIR CORRESPONDING MERCAPTURIC
ACIDS IN RAT RENAL TISSUE SLICES. Toxicology 1993;
79(1):67-79.

Rat kidney slices were produced using a modified version of
a mechanical tissue slicer. The slices were incubated with
various concentrations of L-cysteine conjugates and
mercapturic acids of halogenated alkenes in a submersion
incubation system. The slices showed a time- and
concentration-dependent toxicity to the nephrotoxic
conjugates. Five L-cysteine conjugates were tested.
Comparing the in vitro toxicity data with the in vivo
data for the same compounds results in similar ranking for
the relative nephrotoxicity of the conjugates.

TERATOGENICITY

151
Welsch F. IN VITRO APPROACHES TO THE ELUCIDATION OF MECHANISMS OF CHEMICAL TERATOGENESIS. Teratology 992;46(1):3-14.

This report describes some of the contributions that in vitro methods have made to our progress, however slow, toward understanding mechanisms of chemical teratogenesis. Emphasis is given to the painstaking and time consuming nature of approaches required to elucidate mechanisms. The examples considered are cyclophosphamide, 2-methoxyethanol, and retinoids. Some of the newer methods that take advantage of the recent advances in molecular biology and analytical chemistry have already been applied to studies on teratogenic mechanisms. Prospects for the 1990s are excellent and promise more rapid progress than during the past decade toward unraveling the mysteries of normal developmental biology. That knowledge in turn should be immediately applicable for investigations on developmental toxicant-induced abnormal development.

152
Amacher DE, Stadler J, Schomaker SJ, Verseil C. THE TESTING OF 8 CODED COMPOUNDS IN THE RAT LIMB BUD MICROMASS AND RAT EMBRYOCULTURE SYSTEMS. Teratology 1992;45(5):506.

Cultured at high density, limb bud mesenchyme cells proliferate and differentiate into chondrocytes. In this study, drug-associated inhibition of alcian blue uptake by cartilage proteoglycans in spot cultures of limb bud cells prepared from 13 day rat embryos was used to assess teratogenic potential in vitro following 48 hour exposure to each of 8 coded compounds. These were: acetylsalicylic acid, isoniazid, penicillin G, saccharin, vincristine sulfate, 6-aminonicotinamide, retinoic acid, and amarantha. Following drug removal, cultures were incubated another 96 hours, then cells were fixed and stained with 0.5% alcian blue. Bound dye was extracted and quantitated. In parallel cultures, cell viability was measured by neutral red uptake and protein content was assayed by the BCA method. Except for retinoic acid and vincristine, the maximum test concentration was 1000 ug/mL. Inhibitions greater than or equal to 50% were noted. When the same 8 compounds were tested in a 24-hour embryo-culture model, dysmorphogenesis was evidenced. For 5 of the other chemicals, little or no toxicity was noted up to the maximum test concentration in either model. Based on the results, these complimentary models can detect the same potential morphogenetic effects of potential teratogens in vitro. Their use in parallel should increase the power to detect potential teratogens in

vitro.

153
Laschinski G, Vogel R, Spielmann H. CYTOTOXICITY TEST USING BLASTOCYST-DERIVED EUPLOID EMBRYONAL STEM CELLS: A NEW APPROACH TO IN VITRO TERATOGENESIS SCREENING. Reprod Toxicol 1991;51:57-64.

To develop a mammalian in vitro system for teratogenicity testing, cytotoxicity of xenobiotics was evaluated in pluripotent euploid embryonal stem cells (ESC) derived from mouse blastocysts. The dimethyl-thiazol-diphenyl tetrazolium bromide (MTT) assay was the most appropriate test system for cytotoxicity determinations with ESC. Only compounds that do not require metabolic activation were selected for testing from the database for validation of in vitro teratogenesis assays by Smith et al. Results obtained with ESC were compared to corresponding data from fibroblasts from day-14 mouse embryos to detect differences in sensitivity between undifferentiated and differentiated cells. ESC showed a higher sensitivity to known teratogens than fibroblast cultures, which allows calculation of a sensitivity ratio of "adult" cells (differentiated fibroblasts) to embryonal cells (undifferentiated ESC) in a mammalian system similar to the hydra assay. Although some xenobiotics had to be classified as false negatives in our system, the ESC cytotoxicity assay holds promise as a new in vitro screening assay in teratology.

154
Thomas T, Luchtel DL, Faustman EM. TERATOGENIC EFFECTS OF RETINOIC ACID IN AN IN VITRO RAT WHOLE EMBRYO CULTURE SYSTEM. Toxicologist 1992; 12(1):333.

Retinoic acid has been identified as a teratogen in both animal and human investigations. The aim of this study was to conduct a qualitative and quantitative analysis of the morphological, histological, and cellular changes caused by the all-trans metabolite of retinoic acid (RA). To accomplish this aim we utilized the whole rodent embryo culture system with treatment concentrations ranging up to 3.0 uM RA. Qualitative analysis involved scanning electron (SEM) and light microscopy (LM) studies. Quantitative analysis included flow cytometry and the trypan blue exclusion cytotoxicity assessment. The authors conclude that all-trans retinoic acid caused many changes at the morphological, tissue, and cell level, including increased cell death, changes in excellular matrix, and continued neural crest cell adhesion. However, there was not a

significant change in the cell cycle of cells from treated embryos when compared to controls.

155
Bournias-Vardiabasis N, Huh KW, Hopkins K. EVALUATING TERATOGEN EXPOSURE UTILIZING HUMAN HEAT SHOCK PROTEIN 27 CDNA. Teratology 1992; 45(5):498-9.

A molecular based in vitro teratogen assay has been developed utilizing human chorionic villi cells and a heat shock (stress) hsp 27 DNA probe. It has already been established in Drosophila embryonic cultures and in a number of mammalian systems, that exposure of embryonic cells to teratogens results in specific induction of a subset of heat shock proteins. In this project, the authors investigated the induction of hsp 27 mRNA after teratogen exposure by slot blot hybridization analysis. The hsp 27 probe is biotinylated utilizing a nick translation kit, then total RNA isolated from chorionic villi cells is slot blotted onto nitrocellulose paper using an S & S slot blot apparatus. The nitrocellulose is then probed with the biotinylated probe and visualization of the mRNA-DNA hybrid is accomplished with a standard SA-AP protocol. It is hoped that successful development of this assay can eventually be applied at the clinical level and allow for the first time, the ability to assess teratogen exposure in the developing embryo. Such development would be particularly helpful to pregnant women exposed to environmental contaminants at work, women who consume alcohol while pregnant and even those who may have used a hot tub.

156
Hopkins K, Bournias-Vardiabasis N. THE EFFECTS OF TERATOGENS ON SEROTONIN SYNTHESIS. Teratology 1992; 45(5):480.

An in vitro teratogen assay has been developed utilizing Drosophila embryonic cell cultures and immunohistochemical techniques. It has previously been established that inhibition of differentiation, cell-to-cell communication and cell migration are all operating mechanisms of teratogenesis. The morphological endpoints used in assessing the teratogenic response of this assay involve identifying the inhibition of neuron

and/or muscle differentiation, induction of heat shock proteins, and inhibition of normal neurotransmitter levels. This project investigates the effects of suspected neuroteratogens on levels of serotonin expression in

Drosophila embryonic cell cultures. A chemical is classified as eliciting a teratogenic response if it results in a statistically significant reduction (greater than 50%) in the number of positive neuronal clusters when compared to the control. Results so far suggest that the Drosophila assay is capable of accurately establishing whether the particular agent tested can act as a teratogen by a variety of appropriate endpoints (morphological, biochemical, molecular). It is hoped that this assay can be used not only as a teratogen screen, but also in mechanistic studies of abnormal development, gene involvement in teratogenic resistance, and the possible role of heat shock proteins in preventing birth defects.

157

Asai M, Faber W, Neth L, di Sant'Agnese PA, Nakanishi M, Miller RK. HUMAN PLACENTAL TRANSPORT AND METABOLISM OF ALL-TRANS RETINOIC ACID IN VITRO. Placenta 1991; 12(4):367-8.

All trans-retinoic acid (TRA), which is a natural form of vitamin A, is known to be both a morphogen and a teratogen. The 4-oxo metabolite (4oxoTRA) is also teratogenic in mice. The current investigations were undertaken to determine firstly how TRA and 4oxoTRA are transported across the human placenta, secondly, whether the placenta may control this transport of TRA by metabolizing and binding these retinoids, and thirdly whether the retinoids at pharmacologic concentrations are toxic to the placenta. A human term placental lobule was dually perfused using the methods of Miller et al (1985) with an M199 medium without any vitamin A. TRA and its metabolites were separated and quantitated via HPLC. The results described demonstrate that the human placenta can modulate the transfer and metabolism of the endogenous retinoid, TRA, at pharmacologic doses by producing putative teratogenic metabolites.

158

Maguire C, Regan CM. IN VITRO SCREENING FOR ANTICONVULSANT-INDUCED TERATOGENESIS: DRUG ALTERATION OF CELL ADHESIVITY. Int J Dev Neurosci 1991; 9(6):581-6.

Anticonvulsant-induced alteration in C6 glioma cell adhesivity has been evaluated in two independent in vitro assay systems. A centrifugal shear assay was employed to determine drug-induced change in cell-substratum adhesivity. Valproate and clonazepam were found to significantly increase cell-substratum adhesivity when cells were cultured

at concentrations which were within twice their therapeutic plasma level. A second assay evaluated change in affinity for concanavalin A lectin coated surfaces to determine change in cell surface glycoconjugate expression. Valproate and clonazepam and, to a lesser extent, diazepam significantly decreased drug-exposed C6 glioma cell affinity for concanavalin A lectin coated surfaces. Valproate and clonazepam had approximate IC50 values of 0.75 mM and 75 microM, respectively. The findings are compared and discussed in relation to those obtained with an anti-proliferative assay which has been suggested to predict teratogen potential.

159
Schumacher GH. FINDINGS AND POSSIBILITIES IN EXPERIMENTAL TERATOLOGY. Teratology 1991; 44(6):3B.

Congenital malformations are caused by complicated interactions between genetic and environmental factors. The catalogue of Shepard (1986) contains more than 900 agents with teratogenetic effects in laboratory animals. In human less than 30 agents are known with corresponding actions. The use of in vivo-experiments includes the maternal metabolism and placental transfer. Therefore, only mammals are privileged be studied for the interactions between mother and fetus. The differences between the various species and man in regard of diaplacentary function lead to completely different results. The increasing number of chemical substances to be tested involve the need of more experimental animals. In view of this problem, the progress made in the field of in vitro techniques suggested the application of culture systems. In vitro-methods have some experimental advantages as compared with teratological in vivo test methods. On the other hand in vitro systems have also their disadvantages. Looking at the spectrum of available in vitro-models, the following classification is recommended. 1. Cultivation of cells after isolation into single cells - Monolayer cultures (ML-cultures) - Organoid cultures, 2. Cultivation of tissues without isolation - Organ culture - "Whole embryo" culture. When evaluating the applicability and usefulness of the in vitro-systems for teratological research it has to be differentiated between scientific and practical aspects as well as aspects related

to public health. The various culture techniques have undoubtedly proved to be very fruitful in scientific research. The difficulties concern the assessment of the teratogenic risk of substances of a still unknown effect as basis for decisions on public health. In vivo experiments

can't be replaced by in vitro systems, but the usefulness of in vitro models as an additional strategy in teratological research has been established.

160
Bechter R, Terlouw GD, Tsuchiya M, Tsuchiya T, Kistler A. TERATOGENICITY OF AROTINOIDS (RETINOIDS) IN THE RAT WHOLE EMBRYO CULTURE. Arch Toxicol 1992; 66(3):193-7.

Structural modifications of the arotinoid molecule RO 13-7410 led to a difference in the teratogenic potencies of more than five orders of magnitude in mice in vivo and in micromass cultures of at embryonic limb bud cells (Kistler et al. 1990). Five of these retinoids were selected and tested in rat whole embryo culture to determine the suitability of this in vitro test system for the identification of potentially non-teratogenic derivatives among this class of chemicals. The highest concentrations of the compounds with no effects (NOAEL) on general conceptus growth, on differentiation and on the frequency of dysmorphogenic embryos in vitro were compared with the lowest effective teratogenic doses in vivo (LOAEL) or with the concentrations leading to 50% inhibition of limb bud cell differentiation (IC50) in vitro. The types of dysmorphogenesis in vitro were those typical for retinoids, and for the most part resembled the malformations found in vivo. The authors concluded that the whole embryo culture system is a useful tool for the preliminary testing of retinoids.

TISSUE CULTURE
161
Moody RP, Nadeau B. AN AUTOMATED IN VITRO DERMAL ABSORPTION PROCEDURE: III. IN VIVO AND IN VITRO COMPARISON WITH THE INSECT REPELLENT N,N-DIETHYL-M-TOLUAMIDE IN MOUSE, RAT, GUINEA PIG, PIG,HUMAN AND TISSUE-CULTURED SKIN. Toxicol in Vitro 1993;7(2):167-176.

Cross-species in vitro dermal absorption tests were conducted with the 14C-ring-labelled insect repellent, DEET (N,N-diethyl--toluamide), dissolved in acetone and applied to skin sections (0.5 mm) from a dermatome at a dose rate of about 30 mug/cm2. Skin permeation was determined using an automated in vitro dermal absorption procedure, and was calculated from the percentage recovery of 14C-activity in the receiver solution. Listed in decreasing order, the total percentage of in vitro dermal absorption obtained by 48 hr post-exposure for the six skin types n = 4) was: 36 : 27.5% (rhino mouse), 28 : 4.2% (human), 21 : 2.2% (rat), 15 : 0.8%

(pig), 13 : 9.6% (tissue cultured Testskin) and 11 : 1.4% (hairless guinea pig). Lag times for DEET in vitro dermal absorption in the six skin types ranged from 0.6 hr (human) to 1.9 hr (rat). The 14C-activity recovered in soapy water rinses of the skin specimens at 24 hr post-exposure ranged from 4% (rat) to 18% (mouse). The percentage recovery in methanol skin washes, skin digests, and of 14C-volatiles collected in air traps at 48 hr post-exposure are reported. The total mass balance recovery ranged from 70% (Testskin) to 93% (human). Total mass balance recovery was 84 : 9.2% and 108 : 2.9% for rats and guinea pigs, respectively. In summary, the in vitro data underestimated the dermal absorption observed in vivo and tentative explanations for this lack of agreement are discussed.

162
Yonemoto J, Kawagoe A. LIMB BUD CELL CULTURE FOR IN VITRO TERATOGEN SCREENING. Environ Sci (Tokyo) 1992; 1(3):127-35.

A validation study of rat embryo limb bud cell cultures (LBC) was conducted using metals and related compounds known to be teratogenic to mammalian experimental animals. These compounds are arsenite, arsenate, cadmium, mercuric mercury, methylmercury, indium, lithium, and nickel. Retinoic acid (all-trans) was used as positive control. Fifty percent inhibition concentration for cell proliferation (IP50), 50% inhibition concetration for differentiation (ID50), and the ratio of IP50 to ID50 (P/D ratio) were obtained from LBC. These parameters were compared with available in vivo data, i.e., LD50, lowest published toxic dose for reproduction (TDL0), and the ratio of LD50 to TDL0 (A/D ratio). Among site-specific teratogens, i.e., arsenite, arsenate, cadmium, and methylmercury, P/D ratios were >3 except for methylmercury, while P/D ratios were around 1 among nonteratogenic compounds. It is possible to use LBC to prescreen site-specific teratogenic metal compounds using the criteria that the P/D ratio is >3 and/or a very low ID50 (submicromole level) (specific inhibitor for cell differentiation), or very low P/D ratio (specific inhibitor for cell proliferation). However, it is difficult to segregate nonspecific teratogenic metal compounds from nonteratogenic ones. Therefore, LBC was shown to be useful for metals and related compounds for ranking the priority for further teratogenicity tests, but not always for prescreening teratogenic metal compounds.

VALIDATION TESTS
163

Balls M. THE DEVELOPMENT AND VALIDATION OF IN VITRO TOXICITY TESTS. Anim Cell Technol Eur Soc Anim Cell Technol Meet, 11th;1992;701-12. (18 REFS)

No abstract.

164
Sivak JG, Stuart DD, Herbert KL, Van Oostrom JA, Segal L. OPTICAL PROPERTIES OF THE CULTURED BOVINE OCULAR LENS AS AN IN VITRO ALTERNATIVE TO THE DRAIZE EYE TOXICITY TEST: PRELIMINARY VALIDATION FOR ALCOHOLS. Toxicol Methods 1992; 2(4):280-94.

The ocular lens is a cellular structure that is derived from the embryological surface ectoderm, the same source as that of the epithelium of the cornea and conjunctiva. The physical parameters of the lens are sensitive to factors affecting single cells. In contrast to cultured corneal cells or excised corneas, the lens maintains its refractive function in vitro. Whole lens organ culture (using lenses from abattoir sources) accompanied by both refractive and scatter (relative transmittance) measurements may be an effective toxicological assay. The authors developed an automated scanning laser system designed to monitor the focal length (spherical aberration) and transmittance of the cultured ocular lens during in vitro research. The validity of this approach was studied by examining the effects of five alcohols of 0.5 to 10% concn. (1-butanol, hexanol, Butyl cellosolve, cyclohexanol, and 2-propanol) on the optical quality of the cultured bovine lens. Preliminary comparison with the standard Draize (in vivo) test scores indicates that this system provides a scaled measure of lens damage that compares favorably with standard Draize scores for the compounds included in this study.

165
Dudley DJ, Hatasaka HH, Branch DW, Hammond E, Mitchell MD. A HUMAN ENDOMETRIAL EXPLANT SYSTEM: VALIDATION AND POTENTIAL APPLICATIONS. Am J Obstet Gynecol 1992; 167(6):1774-80.

An in vitro explant system to study the regulation of prostaglandin production by human endometrium is described. Segments of late-luteal-phase endometrium were obtained aseptically at the time of endometrial sampling. The endometrium was cut into pieces (1 mm3) and applied to the polycarbonate membrane of tissue-culture- well inserts for 12-well plates (Costar Transwell cell culture chamber inserts, 0.4 mum pore size). After placing the well inserts,

culture medium was carefully applied. The explants were incubated at 37.degree. in 5% CO2 in air, and the culture medium was changed daily. Electron microscopic examination and lactate dehydrogenase determinations of the explants revealed cellular viability for up to 5 days of culture. Endometrial explants responded to treatment with interleukin-1beta and tumor necrosis factor by a concentration-dependent increase in the production of prostaglandin E2. Costimulation of late luteal endometrial explants with interleukin-1beta and progesterone resulted in variable production of prostaglandin E2. This approach to explant methods is simple and provides a flexible model to study the regulation of the production of bioactive substances by human endometrial tissue.

166
Goldberg AM, Frazier JM, Bussick D, Dickens MS, Flint O, Getings SD, Hill RN, Lipnick RL, Renskers KJ, et al. FRAMEWORK FOR VALIDATION AND IMPLEMENTATION OF IN-VITRO TOXICITY TESTS REPORT OF THE VALIDATION AND TECHNOLOGY TRANSFER COMMITTEE OF THE JOHNS HOPKINS CENTER FOR ALTERNATIVES TO ANIMAL TESTING. In Vitro Toxicol 1993; 6(1):47-55.

No abstract.

167
Stern JJ, Thorsell L, Bustillo M, Coulan CB. VALIDATION OF AN EMBRYO TOXICITY ASSAY. Joint Meeting of the American Association of Immunologists and the Clinical Immunology Society, Denver, Colorado, USA, May 21-25, 1993. J Immunol 1993; 150(8 PART 2):157A.

No abstract.

168
Birge WJ, Silberhorn EM. EVALUATION OF A FISH EMBRYO-LARVAL DEVELOPMENT ASSAY USING SIX COMPOUNDS RECOMMENDED FOR IN VITRO TERATOGENESIS TEST VALIDATION. Toxicologist 1992; 12(1):333.

Tests using fish embryo-larval stages are routinely used for monitoring effluent toxicity and for assessing the hazard of aquatic contaminants. Because of similarities in vertebrate development, these tests also have been proposed as screening systems for identifying and ranking teratogens that may affect mammals. In this study the developmental toxicity of seven chemicals, including five drugs, was

assessed using an 8-day fathead minnow embryo-larval assay. Tested were six compounds recommended for in vitro teratogenesis test validation studies. Endpoints included lethality (LC50), teratogenicity (EC50), and growth (length). Teratogenic potency was assessed using a Teratogenicity Index (TI = LC50/EC50). All compounds tested were teratogenic with TI values ranging from 1.5 to 5.0. For most compounds, growth inhibition was a sensitive indicator of developmental toxicity. Study results from mammalian testing and FETAX were in general agreement, indicating the potential usefulness of this assay for identifying teratogens and developmental toxicants of possible human health concern.

169
Schwetz BA, Morrissey RE, Welsch F, Kavlock RA. IN VITRO TERATOLOGY. Environ Health Perspect 1991; 94:265-8.

The purpose of the conference was to reevaluate the need for and use of in vitro teratology assays; to examine the validation process for in vitro tests; and to discuss progress in the validation of in vitro teratology screens. Participants enthusiastically supported further development of short-term in vivo and in vitro systems both as prescreens for developmental toxicity and as experimental systems to explore mechanisms of action of toxicants. The group strongly endorsed the development of an updated reference list ("gold standard") of known developmental toxicants and nontoxicants as essential to further progress in developing and validating prescreening efforts. Independently, an expert group should further evaluate the performance characteristics for a validated prescreen. The limits of usefulness of prescreens for product development, regulatory use, and mechanistic investigations need to be clearly defined. Finally, too few in vitro teratology prescreens have been evaluated under multiple-laboratory conditions with common, agreed-upon test agents to draw firm conclusions regarding the merit and reproducibility of in vitro teratology prescreens. There was general agreement regarding the need to move several of the assays further along the validation pathway, at least using a short list of reference compounds.

170
Harvell JD, Maibach HI. VALIDATION OF IN VITRO SKIN IRRITATION ASSAYS USING HUMAN IN VIVO DATA. In Vitro Toxicol. J Mol Cell Toxic 1992; 5(4):235-239. (18 REFS)

This review discussed the validation of in-vitro dermal

toxicity assays using in-vivo human data. Since the mechanisms involved in producing skin irritation appear to be both diverse and complex, no single parameter emerged as a best predictor of irritation. The availability of a standardized approach to make in-vitro/in-vivo correlations would test a reference set of compounds both in-vitro and in-vivo. Several bioengineering devices were available which allowed for objective and reproducible assessments of skin irritation. The most widely used instrument for measuring transepidermal water loss was the Evaporimeter which determined the vapor pressure gradient by measuring the vapor pressure at two fixed points immediately adjacent to the skin surface. The principle for laser Doppler flow measurements was the ability to detect a frequency shift in a beam of laser light and equate this shift with flow. Chromametry was a reflectance technique which quantified color components of the skin surface. These devices offered the advantages of objective and reproducible assessments of irritancy.

XYZ/MISCELLANEOUS

171

Stammati A, Zanetti C, Pizzoferrato L, Quattrucci E, Tranquilli GB. IN-VITRO MODEL FOR THE EVALUATION OF TOXICITY AND ANTINUTRITIONAL EFFECTS OF SULPHITES. International Symposium on Current Issues with Food Preservatives: Chemico-Technical, Nutritional and Safety in Use Aspects, Rome, Italy, July 3-5, 1991. Food Addit Contam 1992; 9(5):551-560.

No abstract.

172

Schasteen CS, Donovan MG, Cogburn JN. NOVEL IN VITRO SCREEN TO DISCOVER AGENTS WHICH INCREASE THE ABSORPTION OF MOLECULES ACROSS THE INTESTINAL EPITHELIUM. J Controlled Release 1992;21(Jul):49-62. (14 REFS)

The adaptation of an in vitro cell culture model that fulfills the screen criteria for the detection of absorbed marker molecules, particularly proteins and peptides, and provides a rapid, efficient system for the discovery of novel absorption enhancers is described. The absorption screen uses Caco-2 cells, a human derived intestinal epithelial cell line, grown as monolayer cultures on a permeable membrane that separates apical and basal chambers. Known absorption enhancers, such as laureth 9 (polyoxyethylene-9-lauryl ether) increased the transport of both radiolabeled and fluorescent markers. Laureth 9

enhanced the transport of mannitol to a greater extent than the fluorescent markers sulforhodamine 101 and fluorescein isothiocyanate-dextran. The assay has been used as a screen to identify a number of potential absorption enhancers.

173
Rekasi Z, Schally AV. A METHOD FOR EVALUATION OF ACTIVITY OF ANTAGONISTIC ANALOGS OF GROWTH HORMONE-RELEASING HORMONE IN A SUPERFUSION SYSTEM. Proc Natl Acad Sci USA 1993;90(6):2146-9.

To evaluate the endocrine effect of GH-releasing hormone (GHRH) antagonists, a sensitive dynamic in vitro system was developed. The concentration causing 50% inhibition (IC50) of the standard. GHRH antagonist human [N-Ac-Tyr1, D-Arg2]GHRH-(1-29)-NH2 is 4.5.times. 10-8M in the dispersed pituitary cell superfusion system. This value is 11-fold less than that measured in earlier static pituitary cell cultures. This reliable dynamic system is simple, fast, and inexpensive and not only makes it possible to obtain quantitative data on the inhibitory capacity of the antagonists but also provides information about the intrinsic GHRH activity of the analog. The dynamic interactins of the GHRH antagonist, the GHRH receptors, and GH release can also be evaluated by this superfusion system. The pulsatile GH release induced by 10-9M human GHRH-(1-29)-NH2 was inhibited by 2 modes of application, preincubation and simultaneous administration of the GHRH antagonist (10-9-10-6M). The inhibitory effect of the antagonist was dose-dependent, temporary, and of the competitive type. This sensitive dynamic in vitro system appears to be a suitable method for screening the biological activity of various GHRH antagonists and eliminates the drawbacks of static pituitary cell culture.

174
Hellmer L, Bolcsfoldi G. AN EVALUATION OF THE E. COLI K-12 UVRB/RECA DNA REPAIR HOST-MEDIATED ASSAY. I. IN VITRO SENSITIVITY OF THE BACTERIA TO 61 COMPOUNDS. Mutat Res 1992:272(2):145-160. (45 REFS)

Tests were conducted on 61 compounds to evaluate the Escherichia-coli K-12 uvrB/rec/A in-vitro assay. Of the 61 compounds tested, 32 gave positive results. Of the 44

experiments which were positive with or without metabolic activation, ten showed a significant differential reduction of the two strains at one three fold dilution step only, while the remaining gave up to a 100 fold difference. Three

compounds, acetaldehyde, benzene, and urethane, demonstrated significant preferential toxicity toward the DNA repair proficient strain only, either with or without metabolic activation. Of the 49 compounds tested with and without metabolic activation, only benzidine, thioacetamide, and trimethyl-phosphate gave a positive response with activation alone. Two compounds that are generally mutagenic in-vitro gave negative results in the test, benzo(a)pyrene (50328) and cyclophosphamide (50180). The authors concluded that these bacterial strains were suitable for use as indicator organisms for genotoxicity in-vitro and in-vivo.

175

Janik F, Wolf HU. THE CA2+-TRANSPORT-ATPASE OF HUMAN ERYTHROCYTES AS AN IN VITRO TOXICITY TEST SYSTEM ACUTE EFFECTS OF SOME CHLORINATED COMPOUNDS. J Appl Toxicol 1992; 12(5):351-358. (21 REFS)

An in-vitro test for screening chemical induced inhibition of the Ca2+-transport-ATPase of human erythrocyte membranes was discussed. The system was incubated with 20 or 60 microliter aliquots of the test chemical dissolved in dimethyl-sulfoxide for 10 minutes at 30 degrees-C. The concentration of the test agent causing a 50% reduction in Ca2+-transport-ATPase activity (IC50) was determined. The system was evaluated with 23 chemicals including phenol, chlorinated phenols, chlorinated insecticides, other pesticides, paraquat, and chlorophenoxy herbicides. The authors conclude that the IC50s of the tested compounds agree with results obtained in other in-vitro and in-vivo tests. An inhibitory effect on human erythrocyte CA2+-transport-ATPase indicates that the compound is cytotoxic. The assay should be a useful system for screening compounds for cytotoxicity.

176

Garrelds IM, Zijlstra FJ, Tak CJ, Bonta IL, Beckmann I, Ben-Efraim S. A COMPARISON BETWEEN TWO METHODS FOR MEASURING TUMOR NECROSIS FACTOR IN BIOLOGICAL FLUIDS. Agents Actions 1993;38(Spec No, PC89-91).

The current study was undertaken to compare two methods for the efficiency of measuring tumor necrosis factor (TNF-alpha) in biological fluids, which is species undependent, reliable, sensitive, simple and not expensive. We have compared the MTT tetrazolium cytotoxic assay and the 3H-thymidine (3H-TdR) incorporation cytostatic assay for measuring the anti-tumor activity of human recombinant TNF-alpha, of human colonic tissue and of supernatants of in

vitro stimulated human and rat peritoneal macrophages. Two target cell-lines, namely murine myelomonocytic leukaemia WEHI-164- and L-929-transformed murine fibroblast cell-lines, were used in the MTT assay. The L-929 line was also used in the 3H-TdR assay. WEHI-164 was more sensitive than the L-929 cell-line in the MTT cytotoxic assay. Furthermore, the MTT assay was more sensitive to TNF-alpha than the 3H-TdR assay. Both methods can be used for the detection of anti-tumor activity in biological fluids but the MTT cytotoxic method has the advantage of being more sensitive and more simple.

177
Shelby MD, Erexson GL, Hook GJ, Tice RR. EVALUATION OF A THREE-EXPOSURE MOUSE BONE MARROW MICRONUCLEUS PROTOCOL: RESULTS WITH 49 CHEMICALS. Environ Mol Mutagen 1993; 21(2):160-79.

Forty-nine chemicals were tested in a mouse bone marrow micronucleus test that employed three daily exposures by intraperitoneal injection. Bone marrow samples were obtained 24 hr following the final exposure. Twenty-five rodent carcinogens and 24 noncarcinogens were selected randomly from the 44 carcinogens and 29 noncarcinogens used by Tennant et al. (Science 236:933-941, 1987) to evaluate the performance of four in vitro genetic toxicity tests. This study was conducted as part of an effort to assess the ability of the micronucleus test to discriminate between rodent carcinogens and noncarcinogens and to determine its potential role, in combination with other short-term tests, in identifying genotoxic chemicals that present a carcinogenic hazard. Nine chemicals were judged to be positive in the micronucleus test. This relatively low number of positive results, along with published and unpublished results from rodent micronucleus and chromosome aberration assays on several of these 49 chemicals, contributed to the conclusion that a single micronucleus test protocol is not adequate to detect all chemicals capable of inducing chromosomal damage in the bone marrow. However, a combination of two relatively simple assays such as the Salmonella and micronucleus tests can provide important information on the genetic toxicity of test chemicals and may provide guidance on the need for and the nature and extent of future toxicity studies.

178
Reddy JK, Rao MS, Yeldandi AV. PEROXISOME PROLIFERATION: A BIOLOGICAL MARKER FOR TOXICOLOGICAL EVALUATION. J American

College of Toxicology 1992; 11(3):349-352. (35 REFS)

The mechanisms responsible for tissue specificity and species differences in the biological responses to peroxisome proliferators were reviewed and discussed. Studies have indicated that these proliferators are an important group of xenobiotics having therapeutic, societal, and agricultural importance. They induced liver tumors in rodents but did not exert genotoxic or mutagenic effects directly in short term in-vitro test systems. To understand the relationship of xenobiotic induced peroxisome proliferation to the eventual development of liver tumors, a concerted cell biological, biochemical, and molecular biological approach may be needed. Both the early and delayed effects of exposure to these agents were reviewed, along with the usefulness of peroxisome proliferation as a biological marker. The authors suggest that evaluation of the changes in peroxisome volume density in different species after the administration of therapeutic dose levels of a given peroxisome proliferator may be used as a reliable indicator for toxicological evaluation for this class of nongenotoxic chemicals.

179
Chang LW, Daniel FB, DeAngelo AB. ANALYSIS OF DNA STRAND BREAKS INDUCED IN RODENT LIVER IN VIVO, HEPATOCYTES IN PRIMARY CULTURE, AND A HUMAN CELL LINE BY CHLORINATED ACETIC ACIDS AND CHLORINATED ACETALDEHYDES. Environmental and Molecular Mutagenesis 1992;20(4):277-288. (58 REFS)

The ability of trichloroacetic-acid, dichloroacetic-acid, monochloroacetic-acid and the corresponding aldehydes, chloral-hydrate, dichloroacetaldehyde (DCAA) and 2-chloroacetaldehyde (CAA) to induce DNA strand breaks (SB) was investigated in intact rodent liver, in primary cultures of rat and mouse hepatocytes, and in a human cell line using an earlier developed DNA alkaline unwinding assay. The findings indicated that the chlorinated acetic acids and the chlorinated acetaldehydes did not produce DNA SB in rodent liver cells even when administered at levels up to 500 to 1000 times higher than those seen in finished drinking water. Results indicated that the chloroacetic acids lack genotoxic activity in rodent liver and other rodent tissues and cultured cells.

180
Weischer CH, Krisinger J, Karzel K. EFFECTS OF MELLITIC ACID (MA) AND SODIUM FLUORIDE (NAF) ON THE HISTOLOGICAL APPEARANCE OF MURINE FETAL TIBIAE CULTURED IN

VITRO. Histol Histopathol 1986;1(3):303-8.

The aim of this study was to develop a standardized image analysis method for localization and quantitative measurement of calcified structures of murine fetal tibiae cultured in vitro as a completion and verification of previous biochemical studies. The calcified structures of bone stained by von Kossa silver technique and the epiphyseal cartilages showing intensive metachromasia with toluidine-blue staining were converted with grey-value window programs and afterwards the areas of the selected structures were measured. The histomorphological investigations showed that the murine tibiae, incubated for a period of 6 days in a medium with addition of 5 mmol mellitic acid, showed both a significant reduction of calcium deposits and an increase of epiphyseal intercellular cartilage matrix. The tibiae incubated in a medium with addition of 0.5 mmol sodium fluoride significantly showed an increase of calcium deposits in the thickened lamellae of the compacta. These histomorphological results confirm previous biochemical studies.

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Wiebel FJ. DO WE NEED IN VIVO ASSAYS IN CASE OF NEGATIVE IN VITRO TEST RESULTS? BGA Schr 1993; 1/93:25-31. (17 REFS)

No abstract.