1992 No. 3
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.

CARCINOGENESIS

1

Nersessians AK. ACTIVITY OF HUMAN CARCINOGENS IN THE SALMONELLA AND RODENT BONE MARROW CYTOGENETIC TESTS. Mutation Research 1992;281(4):239-243. (23 REFS)

Additional data were presented on the relationship between activity in the Salmonella and rodent bone marrow cytogenetic assays, and human carcinogenicity. Results were provided for the drugs cyclosporin and thiotepa, recently added to the IARC List of Carcinogens, Group 1. Some other human carcinogens were also considered including ethanol, ionizing radiation, information from Soviet studies on asbestos using the modified Ames assay. The additional data supported the earlier conclusion that the greatest concern should center on agents which are carcinogenic for animals, mutagenic in Salmonella and are rodent clastogens. The author also stresses that nongenotoxic rodent carcinogens should not be automatically neglected as possible human carcinogens. Hormonal or immunosuppressive agents which are carcinogenic for animals but inactive in short term tests may also be carcinogenic for humans by acting to produce tumors by other than genotoxic methods.

2

Smit EF, De Vires EG E, Timmer-Bosscha H, De Leij LF H, Oosterhuis JW, Scheper RJ, Weening JJ, Postmus PE, Mulder NH. IN VITRO RESPONSE OF HUMAN SMALL-CELL LUNG-CANCER CELL LINES TO CHEMOTHERAPEUTIC DRUGS; NO CORRELATION WITH CLINICAL DATA. Int. J. Cancer 1992; 51(1):72-8.

Three cell lines derived from small-cell lung carcinoma (SCLC) tumors of patients who had no clinical response after treatment with a multi-drug regimen were compared to 3 cell lines derived from tumors of patients who, upon treatment, showed a complete clinical response. These 2 groups of cell lines were considered to represent the in vitro counterparts of the 2 extremes of the clinical spectrum of sensitivity for chemotherapeutic drugs in small-cell lung cancer. To assess whether the in vivo (in)sensitivity of a tumor to a certain drug regimen is retained in vitro, the cell lines were tested for drug sensitivity using the microliter-well tetrazolium assay and the results were compared with the in vivo data. No correlation was found. Results of in vitro chemosensitivity testing for individual SCLC patients should be interpreted with

caution.

3
Morgan D, Welty D, Glick A, Greenhalgn D, Hennings H, Yuspa SH. DEVELOPMENT OF AN IN VITRO MODEL TO STUDY CARCINOGEN-INDUCED NEOPLASTIC PROGRESSION OF INITIATED MOUSE EPIDERMAL CELLS. Cancer Res 1992;52(11):3145-3156. (45 REFS)

No abstract.

4
Brusick DJ. CARCINOGENICITY: THE USE OF ANIMAL MODELS AND SHORT-TERM PREDICTIVE TESTS. In Vitro Toxic Test, 1992:221-44. (61 REFS)

Substantial time and resources have been devoted during the past 20 year to a search for reliable short-term tests able to identify animal and, presumed, human carcinogens. To date, the efforts have not produced a test or battery of tests which satisfy the necessary requirements. The reasons for this involve several areas: 1) Limited understanding of the full range of critical events involved in cancer initiation and tumor expression. 2) Tests have been developed for some, but not all of the events. 3) Differences in the approaches to interpretation of animal cancer test results and data from short-term tests. 4) Current methods almost assure a lack of concordance. 5) The lack of a validation standard for the rodent model so that an estimate of its false-positive and false-negative responses can be made. 6) Unavailability of in vitro tests which cover both genotoxic and nongenotoxic mechanisms and which truly complement each other across a range of chemical classes. Research activities in the areas of in vitro cell transformation, in vitro cell line development using shuttle vectors and the development of transgenic animal models offer hope that short-tests for carcinogens are not far from a reality.

5
Berube LR, Harasiewics K, Foster FS, Dobrowsky E, Sherar MD, Rauth AM. USE OF A HIGH FREQUENCY ULTRASOUND MICROSCOPE TO IMAGE THE ACTION OF 2-NITROIMIDAZOLES IN MULTICELLULAR SPHEROIDS. Br J Cancer 1992;65(5):633-640.

A system was designed to allow imaging of control and

drug treated multicellular spheroids with a high frequency backscatter ultra-sound microscope. It allowed imaging of individual spheroids under good growth conditions. Since little data were available on cellular toxicity of ultrasound at these high frequencies (80 MHz), studies were undertaken to evaluate effects on cell survival, using a colony forming assay. No toxicity was observed on cell mono-layers subjected to pulsed ultrasound at the intensities used for imaging experiments. Spheroids were also subjected to pulsed ultrasound and no growth delay was observed when exposed spheroids were compared with mock-exposed spheroids. Imaging studies were performed and pictures of untreated spheroids were obtained in which the necrotic and viable regions are clearly distinguishable. When the hypoxic cell cytotoxin 1-methyl-2-nitroimidazole (INO2) was added to the spheroid, dramatic changes were observed in the backscatter signal. The interior viable cells of the spheroid were selectively affected. Changes in the backscatter signal were also observed when the reduction product 1-methyl-2-nitrosoimidazole (INO) was added to spheroids. With INO however, the changes were located at the periphery of the spheroid. The present work demonstrates the potential usefulness of ultrasound backscatter microscopy in following the action of selected drugs in this in vitro tumor model.

6
Romano P, Aresu O, Parodi B, Malacarne D, Castagneto G, Parodi S, Ruzzon T. ANALYSIS AND COMPARISON OF INFORMATION AND DATA RECORDED IN CARCINOGEN-ICITY ND GENOTOXICITY DATABASES. Environ Health Perspect 1991, 96:113-20.

The Interlab Project is a university-industry joint project recently funded by the Italian government as part of the improvement of the Italian research infrastructure; among its short-term goals are the implementation of data banks of biomedical interest and the spread of informatic tools for biomedical research. Results of both long-term assays of carcinogenicity in rodents and short-term in vitro and in vivo tests of genotoxicity are relevant for a wide body of users, ranging from carcinogenesis research laboratories to industries and governmental agencies. The contents of the most known databases have been compared, with respect to a specific compound, to evaluate both the overall reliability of these systems, compared to longer and more complex assessments carried out

manually starting from bibliographic searches, and the level of concordance among them.

7
Koepf-Maier P, Kolon B. AN ORGANOID CULTURE ASSAY (OCA) FOR DETERMINING THE DRUG SENSITIVITY OF HUMAN TUMORS. Int J Cancer 1992;51(1):99-107.

A model for testing chemotherapeutic agents in vitro is based on an organoid culture method which allows human carcinomas to grow in vitro and to maintain many typical in vivo properties, including 3-dimensional architecture, growth of multiple cell types, expression of morphology differentiation and formation of histotypical structures. The preservation of drug sensitivity and resistance under the conditions of the organoid culture assay (OCA) was demonstrated by investigating 3 strains of a human hypopharynx carcinoma which differed by different sensitivity to cisplatin in in vivo conditions. These differences were retained in vitro and the modified neutral-red (NR) assay was esp. suitable for revealing drug-induced cytotoxic damage in OCA. The procedure is proposed for the in vitro testing of cytostatic drugs before they are administered to patients. The OCA seems to be suitable for defining the patterns of drug sensitivity and resistance of individual human carcinomas in vitro within a few days.

8
Morgan D, Welty D, Glick A, Greenhalgh D, Hennings H, Yuspa SH. DEVELOPMENT OF AN IN VITRO MODEL TO STUDY CARCINOGEN-INDUCED NEOPLASTIC PRO-GRESSION OF INITIATED MOUSE EPIDERMAL CELLS. Cancer Res 1992;52(11):3145-56.

Primary newborn mouse keratinocytes were initiated in vitro by the introduction of the v-rasHa oncogeny via a defective retrovirus. Recipient cells produce squamous papillomas and have high proliferation rate in culture medium with 0.05 mM Ca2+, but fail to grow in medium with 0.5 mM Ca2+ which is permissive for growth of malignant keratinocytes. When v-rasHa-keratinocytes were exposed to mutagens in vitro, proliferative foci stained intensely red with rhodamine stain, could be easily quantitated, and readily incorporated bromode-oxyuridine. Dose-response studies with several mutagens indicated that the number of foci increased with concentration to the point where excessive cytotoxicity developed. Mutagens varied in potency for

producing foci. A subset of cell lines derived from foci produced malignant tumors in vivo, while others were not tumorigenic. Analysis of DNA from cell lines and tumors revealed that most tumorigenic cell lines maintained the v-rasHa genome, whereas the viral sequences were deleted in nontumorigenic cell lines. Cells in foci, but not v-rasHa control cells, expressed keratin 13, a marker which is strongly associated with the malignant progression of skin tumors in vivo. This in vitro assay provides a quantitative model to study chemically induced focal neoplastic progression at the cellular level and to identify agents which may be selective for enhancing malignant conversion.

9
Ronai Z, Reinhardt L, Foiles P, Hecht SS, Hoffmann D, Emura M, Riebe-Imre M, Mohr U. IN-VITRO DIFFERENTIATED CLARA CELLS FROM HAMSTER LUNGS AS TOOL FOR MEASURING THE TRANSFORMING POTENTIAL OF TOBACCO CARCINOGENS. 83rd Annual Meeting of the American Association for Cancer Research, San Diego, California, USA, May 20-23, 1992. Proc Am Assoc Cancer Res Annu Meet 1992;33(0):178.

No abstract.

10
Bartsch H, Malaveille C. SCREENING ASSAYS FOR CARCINOGENIC AGENTS AND MIXTURES: AN APPRAISAL BASED ON DATA IN THE IARC MONOGRAPH SERIES. Complex Mixtures and Cancer Risk 1990; H. Vainio, M. Sorsa, and A. J. McMichael, Editors, IARC Scientific Publications No. 104; Lyon, International Agency for Research on Cancer, :65-74. (21 REFS)

No abstract.

CELL CULTURE
11
Gregotti C, Di Nucci A, Costa LG, Manzo L, Sceli R, Berte F, Faustman EM. EFFECTS OF THALLIUM ON PRIMARY CULTURES OF TESTICULAR CELLS. J Toxicol Environ Health 1992;36(1):59-69.

The objective of this in vitro study was to examine the response of mixed cultures of Sertoli and germ cells to treatment with thallium (Tl) at the range of concentrations that, in previous studies, was shown in

vivo to affect reproduction. Cultures were prepared from the testis of Sprague-Dawley rats. Morphological investigations of cell cultures with the allium showed evident loss of germ cells with significant reduction in prepachytene and pachytene spermat-ocytes and changes in the shape of Sertoli cells. The results are in agreement with in vivo studies, in which thallium treatment at comparable exposure levels manifested its earliest toxic testicular effects in Sertoli and germ cells. They also demonstrate the usefulness of the in vitro culture technique to assess toxic testicular damage rapidly.

12
Klinefelter GR, Kelce WR, Hardy MP. ISOLATION AND CULTURE OF LEYDIG CELLS FROM ADULT RATS. Health Effects Research Lab., Research Triangle Park, NC (USA). Reproductive Toxicology Branch. Govt Rep Announce Index (GRA&I), Issue 14, 1992.

A variety of xenobiotics can result in a significant decrease in spermatogensis, sperm motility and fertility, libido, or simply the circulating level of testosterone. The ability to assess the steroidogenic capacity of the Leydig cell is pivotal to a complete characterization of toxicant-induced effects on reproductive function in the male. Previously, it was impossible to conduct definitive studies to identify direct toxicant-induced effects on Leydig cell function and viability since a method to provide viable, highly purified Leydig cell preparation was un-available. The authors describe such an isolation procedure as well as criteria for maintaining Leydig cells in primary culture. A primary culture of Leydig cells which maintains function over time, provides a model for those interested in addressing the more mechanistic issues in Leydig cell toxicology and permits the determination of the reversibility of toxicant-induced effects in vitro.

13
Keenan MJ. COMPARISON OF THE TRANSPORT, STORAGE, AND TOXICITY OF THE TWO SOURCES OF VITAMIN D. U. S. Department of Agriculture/Cooperative State Res Ser. Fedrip Database, National Technical Information Service (NTIS), Springfield, VA, USA.

Objective: To use laboratory rats to compare the blood profiles, storage, and transport of vitamin D

metabolites resulting from dietary vitamin D with those resulting from skin synthesis of vitamin D. To use cultured cell lines to study the uptake of vitamin D and its metabolites. To develop a new in vitro method using cultured cell lines by which toxicity of vitamin D can be assessed. APPROACH: In the proposed study differences in metabolism of the two sources of vitamin D will be investigated and development of a sensitive biochemical technique to assess the possible toxicity of vitamin D at lower levels than those that produce overt symptoms will be attempted. To investigate differences in metabolism of vitamin D obtained from the diet and vitamin D synthesized in skin laboratory rats will either be exposed to an ultraviolet light source to produce vitamin D in their skin, or fed vitamin D in their diet. Toxicity of vitamin D will be assessed using cultured kidney cells incubated with vitamin D in varying amounts and bound to different carrier proteins. Cultured adipocytes will be used to study the uptake of vitamin D and vitamin D metabolites into storage sites. contact US Dept. of Agriculture for

results.

14
Pantazis NJ, Dohrman DP, Luo J, Goodlett CR, West JR. ALCOHOL REDUCES THE NUMBER OF PHEOCHROMOCYTOMA (PC12) CELLS IN CULTURE. Alcohol 1992;9(3):171-180.

No abstract.

15
Van Pelt FNAM, Hassing IGAM, 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. (38 REFS)

No abstract.

16
Walker C, Ginsler J. DEVELOPMENT OF A QUANTITATIVE IN VITRO TRANSFORMATION ASSAY FOR KIDNEY EPITHELIAL CELLS. Carcinogenesis 1992;13(1):25-32. (53 REFS)

No abstract.

17
Janecki A, Jakubowiak A, Steinberger A. EFFECT OF CADMIUM CHLORIDE ON TRANSEPITHELIAL ELECTRICAL RESISTANCE OF SERTOLI CELL MONOLAYERS IN TWO-COMPARTMENT CULTURES: A NEW MODEL FOR TOXICOLOGICAL INVESTIGATIONS OF THE "BLOOD-TESTIS" BARRIER IN VITRO.
Tox Appl Pharmacol 1992, 112(1):51-57. (31 REFS)

The dose dependent effects of 0.75 to 24 micromolar cadmium-chloride (CdCl2) were studied in relation to tight junction status, secretory activity, and viability of immature rat Sertoli cells (Sc) incubated in a two compartment system. Sc isolated from 18 day old Sprague-Dawley rats were exposed to the range of CdCl2 for 4 or 18 hours on day one or five of the 13 day culture. The early exposure tested the effect on tight junction formation and the later tested the effect on formed junctions with stable transepithelial electrical resistance (TER). TER was correlated with Sc secretory activity, cell number, and viability through immunoactive inhibin, DNA content, and MTT test, respectively. CdCl2 effects depended on toxicant concentration and onset and duration of exposure. Results suggested that CdCl2 may interfere with the development and maintenance of Sc tight junctions without affecting secretory activity, cell number, or viability. The authors related their findings to investigation of the mechanism of testicular toxicants which act at the blood/testis barrier.

18
Burnet NG, Nyman J, Turesson I, Wurm R, Yarnold JR, Peacock JH. PREDICTION OF NORMAL-TISSUE TOLERANCE TO RADIOTHERAPY FROM IN-VITRO CELLULAR RADIATION SENSITIVITY.
Lancet 1992;39(8809):1570-1.

The success of radiotherapy depends on the total radiation dose, which is limited by the tolerance of surrounding normal tissues. Since there is substantial variation among patients in normal-tissue radiosensitivity, we have tested the hypothesis that in-vitro cellular radiosensitivity is correlated with in-vitro normal-tissue responses. We exposed skin fibroblast cell lines from six radiation-treated patients to various doses of radiation and measured the proportions surviving. There was a strong relationship between fibroblast sensitivity in vitro and normal-tissue reactions, especially acute effects. Assessment of radiosensitivity could lead to improved

tumor cure rates by enabling radiation doses to be tailored to the individual.

19
Chiang LC, Chiang W, Chang SF, Chen HY, Yu HS. CHARACTERIZATION OF AN IMMORTALIZED HUMAN CELL LINE DERIVED FROM NEONATAL FORESKIN DIPLOID FIBROBLASTS. J Dermatol 1992;19(1):1-11.

A new human skin cell line, designated as CCFS-1/KMC, immortalized from human neonatal foreskin diploid fibroblast cells, has been subcultured successfully in vitro for more than 500 passages. This anchorage-dependent cell line possesses many common features of transformation such as morphological and cytoskeletal changes, hypotriploidy, infinite lifespan, increasing plating efficiency and saturation density, and decreasing serum requirement and population doubling time. Human papillomavirus (HPV) type 18 DNA was detected in the cell line before and after immortalization by the polymerase chain reaction (PCR) method. Tumorigenicity, however, was not demonstrated in vivo. The authors report that these immortalized human fibroblasts derived from neonatal HPV-18-DNA-contained diploid fibroblasts possess double minute chromosomes (DMs), a karyotypic aberration usually found in cancer cells.

CYTOGENETICS
20
Jelmert O, Hansteen I-L, Langard S. ENHANCED CYTOGENETIC DETECTION OF PREVIOUS IN VIVO EXPOSURE TO MUTAGENS IN HUMAN LYPPHOCYTES AFTER TREATMENT WITH INHIBITORS OF DNA SYNTHESIS AND DNA REPAIR IN VITRO. Mutat Res 1992;271(3):289-298.

No abstract.

CYTOTOXICITY
21
Garza-Ocanas L, Hsieh GC, Acosta D, Torres-Alanis O, Pineyro-Lopez A. TOXICITY ASSESSMENT OF TOXINS T-514 AND T-544 OF BUCKTHORN (KARWINSKIA HUMBOLDTIANA) IN PRIMARY SKIN AND LIVER CELL CULTURES. Toxicol 1992; 73(2):191-201.

The present study was undertaken to assess and compare the in vitro cytotoxicity of toxins T-514 and T-544 of

buckthorn using primary cultures of rat hepatocytes and keratinocytes. Cell cultures were exposed to 6-50 muM toxins for 2-24 h. Cytotoxicity was determined by the release of the cytoplasmic enzyme, lactate dehydrogenase (LDH), in culture media, methylthiazoltetrazolium (MTT) redn., and neutral red (NR) uptake. Both toxins were highly hepatotoxic; T-514 was more toxic than T-544. In the skin cell cultures, the toxicity of the toxins was not as severe and was not expressed until 12 h of exposure.

22
Reinhardt CA, Schawalder H, Zbinden G. CELL DETACHMENT AND CLONING EFFICIENCY AS PARAMETERS FOR CYTOTOXICITY. Toxicology, 1982;25(1):47-52. (4 REFS)

No abstract.

23
Carrera G, Melgar J, Alary J, Lamboeuf Y, Martel P. CADMIUM ACCUMULATION AND CYTOTOXICITY IN RAT HEPATOCYTES CO-CULTURED WITH A LIVER EPITHELIAL CELL LINE. Toxicol In Vitro 1992;6(3):201-206.

Cadmium accumulation and cytotoxicity were compared in hepatocytes in primary culture (HPC) or co-cultured (COC) with a rat liver epithelial cell line (RLEC). Cells were exposed for a 15-day period at 0, 0.045, 0.45 and 0.9 muM-Cd in the incubation medium. Cadmium uptake in COC was always linearly Cd dose and time dependent. In contrast, at the two highest doses for RLEC and only at the highest dose for HPC, a plateau in Cd uptake was observed. In viable cells 95% of the intracellular Cd was found in the cytosol, entirely protein bound. In the three types of culture the amount of Cd-metallothionein (MT) complex was proportional to Cd uptake and was in the order: COC > HPC > RLEC. The COC model was found to offer a powerful tool for studying long-term accumulation and cytotoxicity of Cd in vitro at low exposure levels.

24
Zhang SZ, Lipsky MM, Trump BF, Hsu IC. NEUTRAL RED (NR) ASSAY FOR CELL VIABILITY AND XENOBIOTIC-INDUCED CYTOTOXICITY IN PRIMARY CULTURES OF HUMAN AND RAT HEPATOCYTES. Cell Biol Toxicol 1990;6(2):219-34.

NR in the medium was absorbed and concentrated in lysosomes of cultured rat and human hepatocytes. NR

uptake increased with the time of incubation and reached a plateau in 2 h. Uptake was proportional to the concentration of the NR solution and the numbers of viable liver cells. Prolonged culture of hepatocytes increased the numbers of lysosomes, and thus, the dye accumulation. The NR can be extracted from lysosomes for quantity measurement of hepatocyte viability and cytotoxicity of xenobiotics. With this assay, several serum-free media (e.g., Waymouth's, MEM, LHC-8, etc.) were compared for the maintenance of viable hepatocytes in vitro. LHC-8 medium, which is used to grow human

bronchial epithelial cells, best preserved viable rat hepatocytes. The cytotoxic effects of dimethylnitrosamine (DMN) and aflatoxin B1 (AFB1) were examined by NR assay on rat and human hepatocyte cultures and were found to be dependent on dose and time of the exposures. Human hepatocytes were more resistant to the toxicity of both chemicals. Compared with lactate dehydrogenase leakage test, the NR assay was simpler and more sensitive in detecting the viability and cytotoxicity of xenobiotics in primary cultures of hepatocytes.

25
Nguyen HN, Sevin BU, Averette H, Perras J, Hightower R, Ramos R, Donato D, Penalver M. IN VITRO EVALUATION OF A NOVEL CHEMOTHERAPEUTIC AGENT, ADOZELESIN, IN GYNECOLOGIC-CANCER CELL LINES. Cancer Chemother Pharmacol 1992;30(1):37-42.

Adozelesin is a derivative of an extremely cytotoxic compound, CC1065. This entirely new class of drug binds preferentially to DNA and facilitates alkylation reaction. In the present study, the authors used the ATP chemosensitivity assay to compare the cytotoxic potency of Adozelesin with that of common chemotherapeutic agents in ten gynecological-cancer cell lines. Flow cytometry was also used to study its effects on cell-cycle kinetics. The mean concentrations of drugs required to produce a 50% redn. in ATP levels as compared with controls were determined. Adozelesin was 104-107 times more potent than Adriamycin, cisplatin, 5-fluorouracil, and Cytoxan. Cell kinetics studies revealed significant S and G2 blocks such as those previously reported for other alkylating agents.

26
Malmberg M, Slocum HK, Rustum YM. A MODEL FOR

MIMICKING THE PHARMACOKINETICS OF CHEMOTHERAPY DRUGS FOR EVALUATION OF DRUG EFFECTS IN A SOFT AGAR COLONY FORMATION ASSAY SYSTEM. Sel Cancer Ther 1991;7(4):159-64.

Colony formation assay systems are an important part of in vitro drug evaluation. It would be useful if the in vitro drug cytotoxicity could be carried out under conditions mimicking those employed clinically. The authors developed an individual colony formation assay system that would allow monitoring and quantitation of the growth of individual colonies in the presence of a drug under conditions of continuous and/or short-term exposure, after plating of the cells in agarose. The pharmacokinetic profile of four drugs, cytosine arabinoside, Doxorubicin, 5-fluorouracil and 5-fluoro-2'-deoxyuridine in agarose mimicked those that were achieved when these agents were administered in vivo as an i.v. push.

27
Bassi AM, Piana S, Penco S, Bosco O, Brenci S, Ferro M. USE OF AN ESTABLISHED CELL LINE IN THE EVALUATION OF THE CYTOTOXIC EFFECTS OF VARIOUS CHEMICALS. Boll Soc Ital Biol Sper 1991;67(8):809-816.

The HTC heptoma cell line was used as an "in vitro" model to detect the cytoxicity of eighteen chemicals, chosen on the basis of different biological activities and physicochemical characteristics. Two different cytotoxicity assays measuring cell lethality (CS) or inhibition of cell growth (CF) were applied to confluent cell monolayers or to colony-forming cells, respectively. Cells were exposed to the chemicals at doses ranging from 10-6 M to 10-2 M for 24 h. The results indicated a wide range of IC 50 values from as low as 1 muM (potassium dichromate) to a high as 407.5 mM (ethanol), the sensitivity of the CF test being greater than that of the CS test. A battery of cytotoxicity tests could be established in order to offer simple, rapid and economic methods which can be complementary and, in part, alternative to the use of laboratory animals.

28
Sina JF, Ward GJ, Laszek MA, Gautheron PD. ASSESSMENT OF CYTOTOXICITY ASSAYS AS PREDICTORS OF OCULAR IRRITATION OF PHARMACEUTICALS. Fundam Appl Toxicol 1992;18(4):515-21.

The authors have evaluated the use of cytotoxicity assays in vitro as an alternative to predicting ocular irritation potential in animals. Three different measures of cytotoxicity - leucine incorporation into protein, MTT dye redn., and neutral red uptake - were measured in a presumed target cell, corneal epithelial cells from rabbit, as well as in a nontarget cell, V79 (Chinese hamster lung fibroblasts). An IC50 value was determined for each endpoint in one or both target cells for a series of 27 commercially available compounds and 56 in house materials from a variety of chemical classes (carbonitriles, imidazoles, substituted benzenes, arom. acids, peptides, phenols, esters, etc.). Analysis of the data by Spearman rho rank correlation and Pearson's correlation indicated that none of the endpoint-target cell combinations used here accurately predicts in vivo irritation potential for the group of compounds tested. The authors concluded that the measurement of cytotoxicity is of limited value as an alternative assay for the classes of materials studied.

29
Armstrong MJ, Bean CL, Galloway SM. A QUANTITATIVE ASSESSMENT OF THE CYTOTOXICITY ASSOCIATED WITH CHROMOSOMAL ABERRATION DETECTION IN CHINESE HAMSTER OVARY CELLS. Mutat Res 1992;265(1):45-60. (40 REFS)

Chinese-hamster-ovary (CHO) cells were used to test eight chemicals with different ratios of cytotoxicity to clastogenicity in an effort to investigate the utility and limitations of various cytotoxicity indicators. Immediate or delayed cell killing and growth inhibition were measured along with cell cycle perturbations. All compounds were used at concentrations that reduced cell growth at 24 hours by 50% or less. At 24 hours higher aberration yields were found than at 10 hours, even when minimal cell cycle delay was detected by average generation time (AGT) estimates from BrdUrd labeled cells. Cells with multiple aberrations were seen at 24 but not at 10 hours. The mitotic index (MI) suppression often did not correlate with AGT. The authors concluded that the MI had limited value for dose selection. Even weakly active chemicals were detected at a single time without exceeding a 50% growth reduction at 24 hours.

30
Van Luyn MJ A, Van Wachem PB, Nieuwenhuis P, Jonkman

MF. CYTOTOXICITY TESTING OF WOUND DRESSINGS USING METHYL CELLULOSE CELL CULTURE. Biomater 1992; 13(5):267-75.

Wound dressings may induce cytotoxic effects. Newly developed and highly sensitive 7 day Me cellulose cell culture with fibroblasts was used as the test system. Cytotoxicity was assessed by monitoring cell growth inhibition, supported by cell morphology evaluation using light and transmission electron microscopy. The authors tested conventional wound dressings, polyurethane-based films, composites, hydrocolloids and a collagen-based dressing. It was shown that only 5 out of 16 would dressing did not induce cytotoxic effects. The results are discussed in view of the clinical uses with contaminated wounds, impaired epithelization or hypergranulation.

DENTAL TOXICITIES

31
Ding N. IN VITRO TOXICITY EVALUATION OF 12 KINDS OF DENTAL MATERIALS USING A MODIFIED CELL CULTURE TECHNIQUE. Chung Hua Kou Chiang Hsueh Tsa Chih 1991; 26(4):233-6, 254.

No abstract.

32
Wataha JC, Craig RG, Hanks CT. PRECISION OF AND NEW METHODS FOR TESTING IN VITRO ALLOY CYTOTOXICITY. Dent. Mater. 1992;8(1):65-70.

Previous studies have utilized in vitro alloy cytotoxicity tests to evaluate dental casting alloys. The purposes of this study were to: (1) evaluate the precision of the optical densitometer and visual tests previously used, (2) evaluate a new test measuring absorbance of solubilized formazan dyes, and (3) test the correlation between these tests for cytotoxicity. Balb/c 3T3 cells were plated in 24-well culture trays at 25,000 cells/cm2 around 10 types of dental casting alloys (6 samples/alloy) and incubated for 72 h. Cells were histochemically stained with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)/succinate for 2 h, then fixed, washed, and dried. Toxicity was measured by optical densitometer (OD) scanning, visual assessment, and 560-nm absorbance of DMSO-solubilized dyes. The results were analyzed by ANOVA, Tukey intervals, and coefficients of variation

(CV's). All 3 methods ranked alloy toxicities similarly. The solubilization method was most discriminating due to lower CV's. Correlation between densitometer and solubilization methods was excellent. All methods were repeatable and correlated well, but the solubilization method was more precise and discriminating.

DERMAL TOXICITY

33
Botham PA, Hall TJ, Dennett R, Mccall JC, Basketter DA, Whittle E, Cheeseman M, Esdaile DJ, Gardner J. THE SKIN CORROSIVITY TEST IN VITRO: RESULTS OF AN INTER-LABORATORY TRIAL. Toxicol In Vitro 1992; 6(3):191-194.

The collaborative study reported here was performed to evaluate the reliability of the skin corrosivity test in vitro when performed in independent laboratories. Twenty substances were examined in each of three participating laboratories and the results were compared with existing data from standard assays in vivo. These inter-laboratory comparisons demonstrated that the refined skin corrosivity test is a robust and reliable method in vitro for identifying potential skin corrosive substances.

34
Perkins MA, Roberts DA, Osborne R. DEVELOPMENT OF IN-VITRO METHODS FOR USE OF HUMAN SKIN CELL CULTURES FOR SKIN AND EYE IRRITANCY ASSESSMENTS OF AQUEOUS INCOMPATIBLE MATERIALS. 1992 Annual Meeting of the Society for Investigative Dermatology, Baltimore, Maryland, USA, April 29-May 2, 1992. J Invest Dermatol

1992;98(4):638.

No abstract.

35
Shivji GM, Stetsko DK, Sauder DN. EPIDERMAL CYTOKINE GENE EXPRESSION AN IN-VITRO ASSAY FOR IRRITANTS AND ALLERGENS. 1992 Annual Meeting of the Society for Investigative Dermatology, Baltimore, Maryland, USA, April 29-May 2, 1992. J Invest Dermatol 1992;98(4):638.

No abstract.

DEVELOPMENTAL TOXICITY

36

Toraason M, Bohrman JS, Krieg E, Combes RD, Willington SE, Zajac W, Langenbach R. EVALUATION OF THE V79 CELL METABOLIC COOPERATION ASSAY AS A SCREEN IN VITRO FOR DEVELOPMENTAL TOXICANTS. Toxicol in Vitro 1992; 6(2):165-74.

Thirty-eight coded compounds were tested for their effect on intercellular communication in the V79 cell metabolic co-operation assay. Test chemicals were selected from a list of 47 agents recommended for the evaluation of assays in vitro for developmental toxicants. In addition to testing the effects of chemicals on intercellular communication, a separate cytotoxicity assay determined the concentration of each chemical that inhibited clonal expansion of V79 cells. Seven of 29 designated teratogens were positive for inhibition of intercellular communication in the V79 assay. Additional, 4 teratogens and 1 non-teratogen inhibited intercellular communication at only a single concentration or at cytotoxic concentrations and were scored as equivocal. The overall accuracy for correctly identifying teratogens and non-teratogens was 42% when equivocal chemicals were considered negative, and 50% if they were considered pos. in the V79 assay. Thus, despite relatively low accuracy regarding a diverse group of developmental toxicants, chemicals that did inhibit intercellular communication under the present conditions had a high probability of being a teratogen. The low accuracy reported here contrasts with earlier reports on the assay and possible reasons for this are discussed.

EMBRYOTOXICITY

37

Brown-Woodman P DC, Webster WS, Picker K, Ritchie HE. EMBRYOTOXICITY OF XYLENE AND TOLUENE: AN IN VITRO STUDY. Ind Health 1991;29(4):139-152.

No abstract.

38

Oglesby LA, Ebron-McCoy MT, Logsdon TR, Copeland F, Beyer PE, Kavlock RJ. IN VITRO EMBRYOTOXICITY OF A SERIES OF PARA- SUBSTITUTED PHENOLS: STRUCTURE, ACTIVITY, AND CORRELATION WITH IN VIVO DATA. Teratology 1992;45(1):11-33. (28 REFS)

The embryotoxicity of phenol (108952) and 12 parasubstituted congeners was investigated in an in-vitro rat embryo embryotoxicity assay, the results of which were compared to those of a companion in-vivo study. Embryos taken from female Sprague-Dawley-rats were cultured with a number of congeners and, in some cases, with hepatocytes to characterize a congener toxicity range from slight to severe. After a 42 hour culture period, viable embryos were evaluated morphologically. Analysis of specific endpoints was conducted and total DNA was determined from homogenized embryos using the Boer method. Cocultured hepatocytes were generally associated with ameliorated embryotoxicity; however, they were associated with enhancement of phenol induced embryotoxicity. Absence of hepatocytes was related to positive correlations between growth retardation and phenol molar refractivity. Hepatocyte lipophilicity was associated with the potential to induce growth deficits. Two of the tested congeners were toxic in both in-vivo and in-vitro systems. For the remaining congeners, only maternal toxicity 72 hours after dosing was positively correlated with two of the reported in-vitro measures (embryotoxicity for growth and development in-vitro without hepatocytes). Hepatocyte presence was related to both positive and negative correlations between in-vivo developmental toxicity endpoints and in-vitro embryotoxicity. The authors state that better relationships between chemical structure and function may be illustrated through use of potency measures from dosimetry data.

GENOTOXICITY

39

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

The zebra fish 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. The authors developed methods for the culture of cells from blastula-stage diploid and haploid zebra fish embryos, as well as cells from the caudal and pelvic fin, gill, liver, and viscera of adult fish. Zebra fish 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 contg. a high concentration of mammalian serum. Using calcium phosphate-mediated transfection, a plasmid constructed for use in mammalian cells was introduced into zebra fish embryo cell cultures and expressed in a stable manner. The research results indicate that transfection procedures utilized in mammalian systems can also be applied to zebra fish cell cultures, providing a means for in vitro alteration of the genotype and phenotype of the cells.

40
Gu ZW, Whong WZ, Wallace WE, Ong TM. INDUCTION OF MICRONUCLEI IN BALB/c-3T3 CELLS BY SELECTED CHEMICALS AND COMPLEX MIXTURES. Mutat Res 1992;279(3):217-22.

The genotoxicity of benzo[a]pyrene, cyclophosphamide, 2-aminoanthracene, 2-nitrofluorene, nitrosated coal-dust extracts, and cigarette-smoke condensate were tested with the micronucleus assay using an established mammalian cell line. The results showed that all chemicals and complex mixtures studied induced micronuclei in BALB/c-3T3 cells. These results indicate that BALB/c-3T3 cells are capable of activating certain promutagens and procarcinogens. It seems that in addition to cell transformation, the micronucleus assay in BALB/c-3T3 cells without an exogenous activation system may be useful for in vitro studies to detect genotoxic chemicals and complex mixtures.

41
Kirkland DJ, Dresp JH, Marshall RR, Baumeister M, Gerloff C, Gocke E. NORMAL CHROMOSOMAL ABERRATION FREQUENCIES IN PERIPHERAL LYMPHOCYTES OF HEALTHY HUMAN VOLUNTEERS EXPOSED TO A MAXIMUM DAILY DOSE OF PARACETAMOL IN A DOUBLE BLIND TRIAL. Mutat Res 1992;279(3):181-94.

Paracetamol (acetaminophen) has been examined for mutagenic potential in numerous studies: gene mutation tests consistently gave negative results while in vitro chromosomal aberration tests showed equally consistent positive effects. In vivo studies for chromosome breaking activity gave clearly negative, equivocal or weakly positive results. In particular two reports have indicated that human volunteers taking a maximum daily dose of paracetamol (3 x 1000 mg over 8 h) exhibited significantly elevated frequencies of chromatid breaks

in their peripheral lymphocytes 24 h later. A carefully controlled double-blind study in which volunteers were pre-screened for normal liver function was conducted by the authors. All subjects non-smoking and their diet and environmental exposures were controlled during the study. There was no evidence that individuals responded to the clastogenic potential of paracetamol or that a group response may have been masked by non-responders. In conjunction with the recently published results of the NTP bioassay, showing no carcinogenic activity in mice and no carcinogenic activity in rats except an increase of mononuclear cell leukaemia in female rats which is of doubtful relevance, the study presented argues that paracetamol does not pose an unacceptable (if any) genotoxic/carcinogenic risk to man.

42
Kutzman R, Myhr B, Lawlor T, Young R, Murli H. GENOTOXICITY ASSESSMENT OF MIXED OLIGOMERS OF CHLOROTRIFLUOROETHYLENE USING A BATTERY OF IN VITRO AND IN VIVO/IN VITRO ASSAYS. Report; ISS AAMRL-TR-90-050; Order No. AD-A236 082, 1990,90 pp. NTIS, Springfield, VA, USA.

Halocarbon 3.1 oil, a potential hydraulic fluid consisting of mixed clorotrifluoroethylene (CTFE) oligomers, was evaluated in vitro bioassays to assess

its potential genotoxic activity. The assays conducted were the Salmonella/reverse mutation assay, the Chinese hamster ovary/forward mutation assay, sister chromatid exchange and chromosome aberration assay, the BALB/c-3T3 cell transformation assay an in vivo/in vitro unscheduled DNA synthesis assay and S-phase synthesis assay. CTFE oligomers caused a week evaluation in the mutant frequency in the forward mutation assay without metabolic activation and an increase in S-phase DNA synthesis in rat liver. Because CTFE was negative in all other assays, the results of this test battery would predict no genetic risk from CTFE however, the results from the S-phase synthesis assay indicated that the test substances were hepatotoxic.

43
Elliott BM, Combes RD, Elcombe CR, Gatehouse DG, Gibson GG, Mackay JM, Wolf RC. ALTERNATIVES TO AROCLOR 1254-INDUCED S9 IN IN VITRO GENOTOXICITY ASSAYS.

Mutagenesis 1992;7(3):175-7.

A working party was set up by the UK Environmental Mutagen Society to consider alternatives to Aroclor 1254-induced S9 in in vitro genotoxicity assays, with the aims of considering whether a replacement for Aroclor in its role in general screening assays could be readily identified. The working party concluded that there was sufficient support in the literature to justify the use of an appropriate phenobarbital/beta-naphthoflavone regime as an acceptable alternative to Aroclor.

44
Knudsen LE, Petersen L, Wasserman K. INCREASED DNA REPAIR SYNTHESIS IN HUMAN B-LYMPHOCYTES AS COMPARED WITH T-LYMPHOCYTES AFTER EXPOSURE TO GENOTOXIC AGENTS IN-VITRO. 83rd Annual Meeting of the American Association for Cancer Research, San Diego, California, USA, May 20-23, 1992. Proc Am Assoc Cancer Res Annu Meet 1992;33(0):143.

No abstract.

45
Parodi S, Malacarne D, Romano P, Taningher M. ARE GENOTOXIC CARCINOGENS MORE POTENT THAN NONGENOTOXIC CARCINOGENS. Envir Hlth Persp 1991;95:199-204. (14 REFS)

The relative carcinogenic potencies of genotoxic and nongenotoxic carcinogens was examined. Published carcinogenesis data on 975 chemicals tested in rodent bioassays and 2834 chemicals tested for genotoxicity utilizing 76 types of tests were reviewed. Carcinogens that were present in both databases and which had given definite positive or negative results in at least three short term genotoxicity tests and in at least 75% of the available tests (genotoxicity criteria) were identified. In the carcinogenesis database, 492 compounds were identified as carcinogens. Of these, 113 met the genotoxicity criteria. Sixty seven carcinogens were genotoxic and the remaining 46 were nongenotoxic. The mean TD50 of the genotoxic carcinogens was 1.19mg/kg/day. The mean TD50 of the nongenotoxic carcinogens was 52.5mg/kg/day. The authors concluded that among the examined compounds, genotoxic carcinogens are approximately 50 times more

potent than nongenotoxic carcinogens. Short term genotoxicity tests can detect the most potent of many classes of chemical carcinogens.

46
Elliott BM, Combes RD, Elcombe CR, Gatehouse DG, Gibson GG, Mackay JM, Wolf RC. REPORT OF UNITED KINGDOM ENVIRONMENTAL MUTAGEN SOCIETY WORKING PARTY: ALTERNATIVES TO AROCLOR 1254-INDUCED S9 IN IN VITRO GENOTOXICITY ASSAY. Mutagenesis 1992;7(3):175-177.

A working party was set up by the UK Environmental Mutagen Society to consider alternatives to Aroclor 1254 (Aroclor)-induced S9 in in vitro genotoxicity assays, with the aims of considering whether a replacement for Aroclor in its role in general screening assays could be readily identified. The working party concluded that there was sufficient support in the literature to justify the use of an appropriate phenobarbital/beta-naphthoflavone regime as an acceptable alternative to Aroclor.

47
Schultz K, Ghosh L, Banerjee S. NEOPLASTIC EXPRESSION IN MURINE CELLS INDUCED BY HALOGENATED HYDROCARBONS. In Vitro Cell Dev Biol 1992;28A(4):267-272.

The neoplastic expression in mouse embryo fibroblasts exposed to 1,2-dibromoethane and its chloroanalogue, 1,2-dichloroethane in vitro, was examined. Both are known to be highly toxic, mutagenic, and carcinogenic agents. C3H10T1/2 cells treated with these haloalkanes exhibited altered morphology and were selected further by cloning in soft agar. Soft agar clones were found to induce a 100% multitumor occurrence in the nude mouse model. The results suggest that this pair of mutagens have altered the normal phenotype of mouse embryo cells, and that the latter cells became neoplastic. These neoplastic cell lines will be useful as an in vitro model to study the role of genetic changes in the transformation processes induced by halogenated hydrocarbons.

48
Bakale G, McCreary RD. RESPONSE OF THE KE TEST TO NCI/NTP-SCREENED CHEMICALS. II. GENOTOXIC CARCINOGENS AND NON-GENOTOXIC NON- CARCINOGENS. Carcinogenesis 1992;13(8):1437-45.

A physico-chemical carcinogen-screening test was used to measure the rate constants of electron attachment, kes, of 105 chemicals that had been screened in long-term rodent bioassays and short-term in vitro tests by the NCI/NTP. In the ke test, a pulse-conductivity technique is used to generate and monitor the decay of excess electrons that serve as nucleophilic surrogates for the target tissue of rodents. Of the 61 chemicals that had been found to be rodent carcinogens as well as Salmonella mutagens, 36 yield kes that are equal to or greater than the diffusion-controlled ke of carbon tetrachloride and are considered to be positive ke test responses. In contrast, 29 of the remaining 44 chemicals that are putative non-carcinogens and non-mutagens yield kes that are negative ke test responses. These results are combined with the ke responses of 46 non-mutagenic carcinogens and 20 mutagenic non-carcinogens that were reported earlier and are evaluated to determine the degree to which the measure of electron-accepting capacity that ke provides complements or overlaps the electrophilicity or DNA reactivity of chemicals that is indicated by positive mutagenicity responses in the Ames Salmonella tester strains or by positive structural alerts, S/As, of the chemicals. The combined ke test results indicate that the overall predictivity of the ke test is comparable to and complements the Ames Salmonella test and S/As in identifying rodent carcinogens.

49
Mason JM, Langenbach R, Shelby MD, Zeiger E, Tennant RW. ABILITY OF SHORT- TERM TESTS TO PREDICT CARCINOGENESIS IN RODENTS. Annual Review of Pharmacology and Toxicology 1990;30:149-168. (129 REFS)

The study of chemically induced carcinogenesis using short term tests (STTs) in rodents was reviewed. In-vitro and in-vivo genotoxicity assays and assays for nongenotoxic carcinogens were considered. The development of mutagenesis testing was discussed beginning with use of a bacterial mutagenesis assay. Measurements involved gene mutation in bacteria, and gene mutation and chromosome damage in mammalian cells. Details were provided regarding the Ames test and the Chinese-hamster cell systems. Various testing schemes were outlined and limitations of in-vitro STT were noted. In-vivo testing, particularly for cytogenetic

endpoints, was reviewed and associated with target and nontarget tissues as well as a range of tissues. Test design involved consideration of species and sex of laboratory animal as well as exposure schedule and route. Integration of in-vivo genetic toxicity tests into animal toxicity studies was considered advantageous since it reduces the number of animals used, and offers results relevant to humans. Experimental testing designed around the multistage process of carcinogenesis was discussed. Identification of tumor promoters involved the mouse skin system and the rat liver focus assay. Difficulties associated with the development of transformation assays were presented. Other endpoints included hepatic cell proliferation, metabolic cooperation, activation of protein-kinase-C, and aneuploidy.

50
Allavena A, Martelli A, Robbiano L, Brambilla G. EVALUATION IN A BATTERY OF IN VIVO ASSAYS OF FOUR IN VITRO GENOTOXINS PROVED TO BE NONCARCIN-OGENS IN RODENTS. Teratogenesis Carcinog Mutagen 1992; 12(1):31-41.

2-Chlorethanol, 8-hydroxyquinoline, 2,6-toluenediamine, and eugenol, previously found to behave as genotoxins in in vitro systems and as noncarcinogens in rodents, were evaluated for their ability to induce genotoxic effects in vivo. Rats were given by gavage a single or two successive doses equal to one-half the corresponding LD50, killed at different times after treatment, and examined for the following end points: the frequency of both micronucleated polychromatic erythrocytes in the bone marrow and micronucleated hepatocytes (after partial hepatectomy); the in vivo-in vitro induction of DNA fragmentation, as measured by the alkaline elution technique, and of unscheduled DNA synthesis, as measured by autoradiography, in hepatocyte primary cultures. The two latter end points were also evaluated after in vitro exposure of hepatocytes to log-spaced subtoxic concentrations. 2-Chloroethanol, 8-hydroxyquinoline, and eugenol never produced effects indicative of genotoxic activity. The same happened with 2,6-toluenediamine, with the exception of a significant increase over controls in the amounts of DNA damage and repair displayed by hepatocyte cultures obtained from rats given two 1/2 LD50 separated by a 24 h interval. The results, which, apart the above mentioned exception, are in concordance

with the rodent carcinogenicity results, contribute to underline the role of in vivo short-term tests for the detection of potential genotoxic carcinogens.

HEPATOTOXICITY

51

Kapil A, Koul IB. EVALUATION OF ANTI-HEPATOTOXIC POTENTIAL OF NATURAL PRODUCTS BY IN VITRO MICROSOMAL LIPID PEROXIDATION. Med Sci Res 1992;20(12):449-50.

There have been several reports of studies in which the hepatoprotective ability of plant-derived agents has been correlated with their antilipoperoxidant activity. Several other medicinal plants have been alleged to be effective against liver diseases in traditional medicine, but confirmation of their pharmacological activities as well as the search for active principle/s requires adequate models which are rapid, reliable and economical. Assessment of the antihepatotoxic activity of extracts of these plants has so far been conducted using in vivo assay methods, employing either whole animals or primary cultured hepatocytes. However, these in vivo assay methods require large amounts of samples and the successful preparation of intact liver cells by perfusion is, in practice, difficult. In order to establish a chemical model of lipid peroxidation that is appropriate for the primary evaluation of the antihepatotoxicity activity of fractions, the authors have explored the possibility of using microsomal fractions instead of whole animals or primary cultured hepatocytes.

IMMUNOTOXICITY

52

Luster MI, Portier C, Pait DG, White KL Jr, Gennings C, Munson AE, Rosenthal GJ. RISK ASSESSMENT IN IMMUNOTOXICOLOGY. I. SENSITIVITY AND PREDICTABILITY OF IMMUNE TESTS. Fundam Appl Toxicol 1992;18(2):200-10.

We have previously reported on the design and content of a screening battery involving a "tier" approach for detecting potential immunotoxic compounds in mice (Luster et al., 1988, Fundam. Appl. Toxicol. 10, 2-19). This battery has now been utilized to examine a variety of compounds by the NIEHS Immunotoxicology Laboratory, the National Toxicology Program-sponsored laboratories, and by the Cell Biology Department at the Chemical Industry Institute of Toxicology. The database generated from these studies, which consists of over 50

selected compounds, has been collected and analyzed in an attempt to improve future testing strategies and provide information to aid in quantitative risk assessment for immunotoxicity. Studies presented have

established the ability of each of the tests or test combinations in the screening battery to detect immunotoxic compounds. Efforts are currently underway using this database to determine the relationships between these immune tests and susceptibility to challenge with infectious agents or transplantable tumor cells. The relationship between immunotoxicity and carcinogenicity, as well as genotoxicity, was also determined. There was no relationship observed between immunotoxicity and mutagenicity as determined using in vitro genotoxicity tests. The significance of the observations is discussed in terms of the relationship between immunotoxicity tests and biological/toxicological processes concerned with human health (e.g., infectious disease).

53
Wood SC, Karras JG, Holsapple MP. INTEGRATION OF THE HUMAN LYMPHOCYTE INTO IMMUNOTOXICOLOGICAL INVESTIGATIONS. Fundam Appl Toxicol 1992;18(3):450-9.

The xenobiotics acetoxydimethylnitrosamine (ACDMN), acrolein (ACR), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) are potent immunosuppressive agents of the in vitro primary humoral response of murine splenocytes. The focus of these studies was to determine if human lymphocytes could be modulated by direct exposure to these xenobiotics and therefore be used as an in vitro model system for immunotoxicological investigations. The authors compared the profile of activity of these xenobiotics on cultured murine splenocytes (SPLC) and human tonsillar lymphocytes (HTL). The studies reported suggest that HTL can provide a comparable profile of activity as murine SPLC and can therefore be utilized for evaluating the direct immunotoxic potential of certain xenobiotics.

54
Wood SC, Karras JG, Holsapple MP. INTEGRATION OF THE HUMAN LYMPHOCYTE INTO IMMUNOTOXICOLOGICAL INVESTIGATIONS. Fundam Appl Toxicol 1992; 18(3):450-459. (37 REFS)

No abstract.

LUNGS
55
Zhong B, Gu Z, Whong W, Wallace WE, Ong T. COMPARATIVE STUDY OF MICRONUCLEUS ASSAY AND CHROMOSOMAL ABERRATION ANALYSIS IN V79 CELLS EXPOSED TO ETHYLENE OXIDE. Teratog Carcinog and Mutagen 1991;11(5):227-233. (16 REFS)

Induction of micronuclei and chromosome aberrations in hamster lung fibroblasts by ethylene-oxide (75218) was examined. Chinese-hamster-V79 lung fibroblasts were cultured and exposed to 3500, 6900, 13,800, or 27,700 parts per million (ppm) ethylene-oxide for 30 minutes. The cells were examined for chromosome aberrations. Other V79 cell cultures were exposed to 457, 1372, 4115, or 12344ppm ethylene-oxide for 30 minutes. Ethylene-oxide induced significant dose dependent increases in chromosome aberration frequency. The aberrations consisted of chromatid and isochromatic breaks, fragments, minutes, and exchanges. Ethylene-oxide increased the frequency of micronucleated V79 cells; however, only the increase induced by the 12,344ppm exposure was statistically significant. The authors conclude that the chromosome aberration and micronuclei induction data indicate that ethylene-oxide is a clastogen. Chromosome aberration analysis appears to be a more sensitive technique for detecting clastogenicity than micronuclei analysis.

56
Thorsen SA. CALORIMETRY: A NEW QUANTITATIVE IN VITRO METHOD IN CELL TOXICOLOGY. A DOSE/EFFECT STUDY OF ALVEOLAR MACROPHAGES EXPOSED TO PARTICLES. J Toxicol Environ Health 1992;36(4):307-18.

A short-term toxicological test has been developed using a calorimetric method. The metabolic activity, observed as the heat exchange rate, was monitored from alveolar rabbit macrophages in monolayers exposed to different metal and non-metal particles. Calorimetric activity indices and viability indices were introduced, from which toxic effects could be assessed. Manganese dioxide particles were found to be cytotoxic. In contrast, titanium dioxide particles seemed to be harmless. Toxic effects from quartz in the form of increased metabolic activity of exposed cells could be detected by the calorimeter in contradiction to the use of the image analyzer. This latter result supports the

hypothesis that silica particles cause chronic modification of the macrophage function and that this change in the alveolar macrophage function may be the first of a series of processes leading to pulmonary fibrosis.

MECHANISMS OF TOXICITY

57

LEAD ENCEPHALOPATHY IN LIVESTOCK: CELLULAR MECHANISMS OF TOXICITY. U. S. Department of Agriculture/Cooperative State Rer Ser Fedrip Database, National Technical Information Service (NTIS), Springfield, VA, USA (Approx 1992).

Purpose: To develop and characterize bovine astroglial cultures for lead toxicity studies; to quantitate lead uptake, perturbation of intracellular trace metal concentrations by lead, and effects of lead on Na: K ATPase activity in astroglial cultures. APPROACH: Astroglial were cultured from fetal or early postnatal bovine cerebral cortex and characterized by immunocytochemistry. Sublethal toxic lead doses were determined by viability measurements. Lead uptake and intracellular trace metal concentrations were determined by atomic absorption spectrophotometry. Na:K: -ATPase activity was determined spectrophotometrically by monitoring the production of inorganic phosphate. PROGRESS: Astroglia serve as a site of lead (Pb) deposition in Pb-exposed animals. Thus, the potential exists for astroglial function to be impaired by elevated intracellular Pb levels. It

was previously shown that the specific activity of glutamine synthetase (GS), an astroglial enzyme with a key role in glutamate and ammonia metabolism in the brain, is reduced in fetal guinea pigs exposed to low levels of lead. This observation indicates either a direct effect of Pb on astroglia or an indirect systematic effect. Lead treatment reduced the ability of cells to exclude trypan blue in a dose and time-dependent manner. The effects of Pb exposure on GS activity were much more pronounced. The much greater sensitivity in vitro of GS activity than dye exclusion or cell numbers to the low level lead supports a specific enzymatic inhibition.

58

Wilson BW, Seiber JN. ANIMAL, CELL AND ENZYME MODELS

FOR THE DETECTION OF EXPOSURE TO PESTICIDES AND OTHER TOXICANTS. U. S. Department of Agriculture/Cooperative State Res Ser. Fedrip Database, National Technical Information Service (NTIS), Springfield, VA, USA.

Use organophosphate esters (OPs) & other toxicants like carbamates & heavy metals to establish evidence for exposures to toxicants and early warning signs of nerve and muscle disorders, find out the cellular and molecular mechanisms of the toxicities, study treatments to counteract the actions of he agents and devise cell culture systems for studying their toxicity. APPROACH: OPs like parathion, DEF and DFP are used to acutely and chronically treat chickens, quail and other birds, embryo muscle, nerve and liver cultures and their biochemistry and morphology examined for signs of toxicities. Plasma and tissue samples are collected from wild and caged birds and their enzymes examined for evidence of exposure to toxic chemicals. PROGRESS: The potential genotoxicity of the phosphoramidate agent Tabun (GA) was evaluated with several in vitro and in vivo tests. Positive results with the mouse lymphoma assay, Ames test and the in vitro SCE assay led to the conclusion GA was a relatively weak, direct acting genotoxic agent. A three-dimensional chick embryo brain cell reaggregate system was developed for use as a model to study OP toxicity. Experiments on the actions of the neuropathic DFP and the non-neuropathic paraoxon show that OPs rapidly damage nerve cell mitochondria. Ryanodine (RY), an alkaloid that acts on the calcium channel of the sarcoplasmic reticulum and other drugs were used to study the expression of the acetylcholinesterase (AChE) forms of quail muscle in culture. Conclusions Pending.

59
Battula N, Schut HAJ, Thorgeirsson SS. CYTOCHROME P4501A2 CONSTITUTIVELY EXPRESSED FROM TRANSDUCED DNA MEDIATES METABOLIC ACTIVATION AND DNA-ADDUCT FORMATION OF AROMATIC AMINE CARCINOGENS IN NIH 3T3 CELLS. Mol Carcinog 1991,4(5):407-414. (41 REFS)

The metabolic activation and DNA adduct formation of carcinogenic aromatic amines as a result of the actions of cytochrome P4501A2 was studied. Mouse cytochrome P4501A2 DNA was transduced into NIH 3T3 cells by retrovirus mediated gene transfer. The activity of cytochrome P4501A2 was determined by enzyme studies. DNA was isolated using phenol extraction and the

adducts quantified by phosphorus-32 post labeling assay. Exposure of a cell clone determined to have enzymatically active cytochrome P4501A2 to 2-amino-3-methylimidazo(4,5-f)quinoline (IQ) or 2-acetylamino-fluorene (AAF) resulted in an increase in DNA adduct formation as compared to control cells. The pattern of adducts formed in the IQ exposed cell line was almost identical to the pattern seen in DNA from the liver of mice and rats exposed to IQ in-vivo. The authors concluded that the cell system described here is a good model system for determining chemical carcinogenesis in-situ.

MEMBRANES
60
Bara M, Guiet-Bara A, Durlach J. A NEW METHOD OF IN VITRO PRESCREENING EVALUATION OF THE RELATIONSHIP BETWEEN TOXIC AND COMMON METAL IONS. Methods Find Exp

Clin Pharmacol 1992;14(4):311-4.

The human amniotic membrane, an asymmetrical and nonexcitable epithelium with sites differently situated on the fetal and maternal sides, may be considered a model for investigating the relationship between toxic and common metal ions. The method is based on the observation of the ionic transfer across the amnion, estimated by measuring the total ionic conductance Gt from the mother to the fetus and from the fetus to the mother. It is important to note that opposite effects between two ions are not necessarily correlated with antagonism; indeed, pollutants decrease ionic conductance Gt and Mg increases it, but Mg is not an antagonist of all pollutants. To define antagonism between two ions, the Dixon curves theory should be applied. These curves represent the variation of Gt when the concentration of common metal increases (1 mM, 3 mM), while the concentration of toxic metal is maintained constant (3 concentrations of toxic metal are used). The straight lines obtained are either parallel to each other (noncompetitive inhibition), parallel to the x axis (no interaction between common and toxic metals), or the 3 lines intersect at a common point equal to the inhibition constant. At pharmacological doses, there is competitive inhibition (specific antagonism) between Mg and Cd, Zn and Cd, Ca and Cd, and Mg and Pb, and non-competitive inhibition between Mg and Hg. This method may rapidly indicate a membrane interaction between common and toxic metals.

MUTAGENICITY

61

Jung R, Engelhart G, Herbolt B, Jackh R, Muller W. COLLABORATIVE STUDY OF MUTAGENICITY WITH SALMONELLA TYPHIMURIUM TA102. Mutat Res 1992;278(4):265-270. (11 REFS)

No abstract.

62

Speit G, Menz W, Roscheisen C, Koberle B. CYTOGENETIC AND MOLECULAR CHARACTERIZATION OF THE MUTAGENICITY OF CHLORAMBUCIL IN V79 CELLS. Mutat Res 1992; 283(1):75-81.

Chlorambucil (CBC) is used as a chemotherapeutic agent and immunosuppressant. Recently, it could be shown that CBC is considerably more effective than radiation or any chemical investigated to date in inducing high yields of germ-line mutations that appear to be multilocus deletions or other structural changes. We therefore reinvestigated the in vitro genotoxic effects of CBC in V79 cells and characterized induced sister-chromatid exchanges (SCEs), chromosome aberrations and gene mutations by means of cytogenetic and molecular methods. CBC effectively induced chromosome aberrations and SCEs in a dose-dependent manner. The chromosome aberrations found after a 14-h treatment were mainly chromatid-type aberrations. 3-Aminobenzamide (3AB) did not influence the incidence of CBC-induced SCEs and chromosome aberrations. Combined treatment with CBC and caffeine (CAF) strongly increased the frequency of aberrations, but had no effect on the yield of SCEs. CAF at lower concentrations enhanced the production of chromatid breaks and exchange figures while higher concentrations (10(-3) M) caused multiple breaks and pulverized mitoses. These results revealed striking differences in the mutagenic action of an alkylating agent in cultivated cells compared to germ-line cells at the molecular level.

NEUROTOXICITY

63

Veronesi B. IN-VITRO SCREENING BATTERIES FOR NEUROTOXICANTS. Third Meeting of the International Neurotoxicology Association, Salsomaggiore Terme, Italy, July 1-5, 1991. Neurotoxocology (Little Rock) 1992;

13(1):185-195.

No abstract.

64
Nostrandt AC, Ehrich M. DEVELOPMENT OF A MODEL CELL CULTURE SYSTEM IN WHICH TO STUDY EARLY EFFECTS OF NEUROPATHY-INDUCING ORGANO-PHOSPHORUS ESTERS. Toxic Let 1992;60(1):107-114. (17 REFS)

This study was designed to determine if neurotoxic esterase (NTE) inhibition and aging could be measured in a human neuronal cell culture system exposed to organophosphorus esters. Results were compared with those found using the nervous system of the chicken. The human neuroblastoma cell line SY-5Y was exposed to mipafox or one of the four negative controls paraoxon, aldicarb, beta, beta'-iminodipropionitrile (IDPN), and carbachol. The NTE aging assay was conducted to determine the amount of NTE bound to the organophosphorus inhibitor and aged. No compound morphologically altered the SY-5Y cells. No NTE aging or inhibition was seen with paraoxon, IDPN, or carbachol treatment. Aging was initiated 2 to 5 minutes after exposure; most of the inhibited NTE was aged by 10 minutes. The time course and effects on NTE in the SY-5Y cells were similar to those seen using chicken brain tissue. The authors conclude that the SY-5Y model system may be useful in determining mechanisms related to organophosphorus induced delayed neuropathy.

65
Harvey AL. NEUROTOXICITY. In Vitro Toxic Test 1992; 111-29.

A review and discussion with 93 refs. Human neurotoxicol. effects, in vitro neurotoxicity assays, and future developments or in vitro assays are discussed.

66
Sawyer TW, Weiss MT, Unger RJ. ANTICHOLINESTERASE ACTIVITY OF ORGANO-PHOSPHATE NERVE AGENTS IN NEURONAL TISSUE CULTURE. Toxicol In Vitro 1992;6(3):261-266.

Primary chick embryo forebrain culture was examined with respect to its usefulness as a model to study organophosphate (OP) anticholinesterases. The

acetylcholinesterase (AChE) activity of these cultures increased with time in culture and paralleled the development in ovo of this enzyme. The neuronal cells were extremely sensitive to the anticholinesterase effects of OP nerve agents, and experiments with soman indicated that enzyme inhibition was rapid and persistent. The potencies of several OP nerve agents as inhibitors of AChE activity in vitro paralleled their literature-reported toxicities in vivo.

67
McLane JA. COMPARISON OF IN VITRO AND IN VITRO MODELS OF PERIPHERAL NEUROPATHY INDUCED BY TOXINS AND THERAPEUTIC AGENTS. Veterans Administration/Research and Development, 810 Vermont Ave. N.W., Washington, D.C. 20420, United States of America. Fedrip Database, National Technical Information Service (NTIS), Springfield, VA, USA.

Axonal transport is a critical cellular process involved in the development and maintenance of neurones. Traditionally, the process has been studied in in vivo animal models. The authors propose to develop methods which would allow for the detailed study of axonal transport using video-microscopy techniques on cultured neurite-producing cells. The authors hypothesize that such methods will allow them to obtain information regarding the neurotoxicity of drugs and chemicals, and the mechanism(s) by which neurotoxins produce peripheral neuropathy. In the proposed studies they will compare the effects of known neurotoxins (i.e., acrylamide, 2,5-hexanedione, p-bromophenylacetylurea) on fast axonal organelle transport in sciatic nerve axons from animals and in neuroblastoma cell neurites. In vitro cell cultures are already utilized widely to screen for cytotoxicity of chemicals and to test whether chemicals cause cell transformation as a prelude to the development of cancer. If it is found that toxins alter organelle transport in the in vivo and in vitro models similarly, it is suggested that neuron-like cell cultures may have a valuable potential in the study of neuropathogenic mechanisms and in screening chemicals, new drugs or environmental agents for neuropathological activity. Single cell models would not only reduce the number of animals needed in neurotoxicological and neuropathological studies, but should also be more economical in terms of experimental time, space, and resources.

68
Tahti H, Naskali L. THE EFFECTS OF ORGANIC SOLVENTS ON NEURAL MEMBRANE INTEGRAL PROTEIN TESTED IN NEURAL CELL CULTURES. Neurosci Rer Commun 1992;10(2):71-77.

The effects of aromatic hydrocarbons and n-hexane, as well as n-hexane metabolites, on ATPase activities were studied in cells isolated from two different rat neural cell cultures: whole-brain reaggregate cultures and cerebellar granule cell cultures. The activity of the membrane-bound integral protein ATPase was used as a biomarker for the membrane effect of organic solvents. Both total ATPase and Mg2+ activated ATPase were determined. Primary granule cell cultures were established from new-born rat cerebella, and the reaggregate cultures were prepared from fetal rat brains. The cells were cultured in modified minimum essential medium (MEM), and harvested for 1-hour solvent treatment in incubation mixtures (at 37~ C), after which the enzyme activities were determined. The aromatic hydrocarbons benzene, toluene, styrene and xylene had an enzyme-inhibiting effect depending on their lipid solubilities. The metabolites of n-hexane (2-hexanone and 2-hexanol) had a more marked enzyme-inhibiting effect than n-hexane itself.

69
Atterwill CK, Johnston H, Thomas SM. MODELS FOR THE IN-VITRO ASSESSMENT OF NEUROTOXICITY IN THE NERVOUS SYSTEM IN RELATION TO XENOBIOTIC AND NEUROTROPHIC FACTOR-MEDIATED EVENT. Third meeting of the International Neurotoxicology Association, Salsomaggiore Terme, Italy, July 1-5, 1991. Neurotoxicology (Little Rock) 1992;13(1):39-53.

No abstract.

70
Veronesi B. IN VITRO SCREENING BATTERIES FOR NEUROTOXICANTS. Neurotoxicology 1992;13(1):185-195. (39 REFS)

No abstract.

71
Atterwill CK, Johnston H, Thomas SM. MODELS FOR THE IN

VITRO ASSESSMENT OF NEUROTOXICITY IN THE NERVOUS SYSTEM IN RELATION TO XENOBIOTIC AND NEUROTROPHIC FACTOR-MEDIATED EVENTS. Neurotoxicology 1992;13(1):39-53. (55 REFS)

No abstract.

72
Halks-Miller M, Fedor V, Tyson CA. OVERVIEW OF APPROACHES TO IN VITRO NEUROTOXICITY TESTING. J American College of Toxicol 1992;10(6):727-736. (33 REFS)

No abstract.

OCULAR TOXICITY

73
Cornelis M, Dupont C, Wepierre J. PREDICTION OF EYE IRRITANCY POTENTIAL OF SURFACTANTS BY CYTOTOXICITY TESTS IN VITRO ON CULTURES OF HUMAN SKIN FIBROBLASTS AND KERATINOCYTES. Toxicol in Vitro 1992;6(2):119-28.

The cytotoxicity of surfactants was evaluated on cultures of human skin fibroblasts and keratinocytes to predict their eye irritancy potential taking into account immediate cytotoxicity after 2 h of incubation and delayed cytotoxicity 24 h after such incubation. The immediate cytotoxicity ranking of the surfactants, evaluated by MTT or neutral red assay after 2 h of exposure in min. Eagle's medium (MEM) without or with 10% fetal calf serum (FCS), was identical for both cell types. Keratinocytes were less sensitive than fibroblasts to all surfactants apart from Tween; however, cytotoxicity ranking remained the same for both cell types. No correlation was found between EC50 values of immediate or delayed cytotoxicity, under various experimental conditions, and ocular irritation scores in vivo. Brij surfactants, reported not to be irritant in vivo, showed a high degree of toxicity in the cell culture systems; however, a good correlation was found when analysis was carried out excluding these surfactants. In contrast, there was excellent correlation between CRR and ocular irritation scores in vivo, for all surfactants tested, including Brij.

74
Smyth RJ, Moore JJ, Shapourifar-Tehrani S, Lee DA. THE EFFECTS OF 5-FLOURO-URIDINE, 5-fLOURODEOXYURIDINE, AND 5-FLOURODEOXYURIDINE MONO-PHOSPHATE ON RABBIT TENON'S

CAPSULE FIBROBLASTS IN VITRO. J Ocul Pharmacol 1991; 7(4):329-38.

Inhibition of rabbit subconjunctival fibroblast attachment and proliferation by 5-fluorouridine (FUR), 5-fluoro-2 deoxyuridine (FUdR), and 5-fluoro-2-deoxyuridine-5-monophosphate (FdUMP), was determined by [3H]adenosine uptake, cell counting, and colorimetric assays for the concentration range of 1000 to 0.0001 mug/mL over a 9 day period. The mean 50% inhibitory doses against proliferation were calculated for each assay. Rabbit fibroblast attachment was not inhibited at any drug concentration by either FUR, FUdR, or FdUMP. For rabbit fibroblast proliferation, FUR was found to be 10-100 fold more potent than FUdR and FdUMP. When comparing the human and rabbit cells, the unpaired t-test analysis showed no consistent difference of the ID50s for FUR, FUdR or FdUMP. Rabbit ocular fibroblasts may be useful in modeling the proliferation of human ocular fibroblasts. These in vitro results may be useful for predicting optimal drug dosages for future in vivo testing of these drugs.

75
Gautheron P, Dukic M, Alix D, Sina JF. BOVINE CORNEAL OPACITY AND PERMEABILITY TEST: AN IN VITRO ASSAY OF OCULAR IRRITANCY. Fundam Appl Toxicol 1992;18(3):442-9.

Most of the published in vitro tests of ocular irritancy investigate a single parameter, generally cytotoxicity, using different cell types in culture. Although good correlations with in vivo data have been reported by some investigators, many of these studies examined only limited classes of products, mainly surfactants and cosmetic ingredients. To predict the irritant potential of compounds in development and process intermediates (which include a wide variety of chemical classes with variable physical characteristics), an assay which would allow great flexibility was needed. A recently published bovine model of corneal opacity was appropriate for this purpose and therefore investigated. The method was substantially modified and extended to study, in the same assay, two important components of irritation, i.e., opacity and permeability. In combination, the measurement of the two endpoints appeared to be sufficient to accurately predict ocular irritancy. In short, the bovine corneal opacity and permeability assay allows investigation of two important components of eye irritation, in a one-day experiment, using a bovine ocular tissue. It represents a useful approach to assess ocular irritation at

least for our needs.

PULMONARY TOXICITY
76
Sarlo K, Clark ED. A TIER APPROACH FOR EVALUATING THE RESPIRATORY ALLERGENICITY OF LOW MOLECULAR WEIGHT CHEMICALS. Fundam Appl Toxicol 1992;18(1):107-14.

A multi-level approach for evaluating low molecular weight chemicals as respiratory sensitizers was proposed. The approach involves four levels of testing that utilize both in vitro and in vivo methods. Tier 1 evaluates structure-activity information to determine if the chemical can covalently modify carrier molecules. It also includes a literature search to determine if the compound belongs to a family of chemicals that has been reported to induce hypersensitivity. Tier 2 tests the chemical's potential to haptenate carrier molecules (i.e., protein) under in vitro conditions. Positive results in Tiers 1 and 2 lead to testing in a guinea pig injection model to assess chemical immunogenicity (Tier 3). A positive result at this level leads to testing in a guinea pig inhalation model to address questions about relevant routes of chemical exposure and allergenicity (Tier 4). Tier 4 results are used in determining safe chemical exposure levels. The data indicate that this approach can detect chemical allergens and can be used to characterize them as moderate or strong respiratory sensitizers.

REPRODUCTIVE TOXICITY
77
Chou K. AUTOMATED IN-VITRO TEST SYSTEM FOR CHEMICAL TOXICITY IN MAMMALIAN SPERM. U. S. Department of Agriculture/Cooperative State Res Ser Fedrip Database, National Technical Information Service (NTIS), Springfield, VA, USA.

The overall objective of the proposed studies are to compare the relative toxicity of three organophosphates in sperm metabolic activity with their relative effects on intracellular calcium concentration and sperm motility. The specific aims were: 1) to determine whether organophosphate insecticides alter sperm motility or velocity. 2) To determine whether organophosphate insecticides alter capacitation related increases in intracellular calcium concentration in the sperm. 3) To examine effects of organophosphate

insecticides on energy utilization in capacitating sperm. APPROACH: Ejaculated boar sperm was treated with three organophosphate insecticides, and assessed for the parameters listed as specific aims. These parameters will be related to one another and to the relative toxicity of the three compounds. (Contact authors for results.)

78
Goldman JM, Cooper RL. USE OF PERFUSION TO EVALUATE HORMONAL RELEASE IN VITRO FROM RAT PITUITARY AND HYPOTHALAMIC TISSUE. Govt Rep Announce Index (GRA&I), Issue 12, 1992.

The use of in vitro procedures in reproductive toxicology has permitted a direct assessment of hormonal release from isolated tissue and a means by which to determine potential sites of toxicant insult. The present report describes a perfusion procedure that can be used to evaluate stimulated or baseline hormonal secretion from pituitary and brain hypothalamic tissue. The implementation and utility of various combinations of tissue stimulation are presented, along with a discussion of the relative advantages and limitations of perfusion as an experimental approach. (Also see report in Methods in Reproductive Toxicology, 1992 or 1993.)

SKIN
79
Burnham K, Rahman M. EFFECTS OF PETROCHEMICALS AND ULTRAVIOLET RADIATION ON EPIDERMAL IA EXPRESSION IN VITRO. J Toxicol Environ Health 1992;35(3):175-85.

We previously demonstrated that combined treatment of mice with crude oil and longwave ultraviolet radiation (UVA) led to the depletion of IA-positive cells from the epidermis. In the present study, we have developed an in vitro screening assay for combined effects of purified petrochemicals and UVA on epidermal IA and Thy-1 expression. This method involves removal of skin from donor mice prior to treatment with chemicals and UVA (20,000 J/m2), followed by in vitro culture and subsequent immunoperoxidase staining. In this study, a complete correlation was observed in terms of IA-positive cell density among similarly treated cultured skin and live mice. The in vitro assay developed for this study should prove to be a valuable tool for the screening of a wide variety of

chemicals for contact photosensitizing activity.

80
de Lange J, van Eck P, Elliott GR, de Kort WL, Wolthuis OL. THE ISOLATED BLOOD-PERFUSED PIG EAR: AN INEXPENSIVE AND ANIMAL-SAVING MODEL FOR SKIN PENETRATION STUDIES. J Pharmacol Toxicol Methods 1992;27(2):71-7.

To overcome most of the disadvantages of current models to investigate percutaneous penetration of drugs or toxic substances, a model is proposed here based on the isolated pig ear, which is obtained at the slaughterhouse, and perfused with oxygenated blood from the same pig. To determine the viability of the preparations, we measured glucose consumption and lactate production as metabolic parameters, Na+ and K+ ions, as well as lactate dehydrogenase activity in blood as markers for cell damage, whereas vasomotor reactivity was assessed by administering noradrenaline and isoxsuprine. After 60 min of equilibration, only insignificant changes in these parameters were observed during the subsequent 3-hr test period. It was concluded that the technique offers an easy to handle, cost- efficient, and animal-saving model for skin penetration studies that lacks most of the disadvantages of existing models.

81
Schaefer H, Filaquier C. SKIN METABOLISM. Pathol Biol 1992;40(2):196-204.

The skin, one of the organs which accounts for the largest proportion of total body weight, was long viewed only as a passive physical barrier between the body from the environment. Over the last ten years, many studies have demonstrated significant metabolic processes in the skin, due in particular to the effects of enzymes which are located mainly in the epidermis. This skin metabolism has a marked effect on percutaneous penetration of xenobiotics. Enzyme activities detected in the skin and their location in the various skin layers are discussed, as well as the different in vitro and in vivo models for studying skin metabolism. Pharmacologic or toxic effects of active ingredients or their metabolites may be associated with the nature and magnitude of transformations of xenobiotics in the skin (first pass effect).
The impact of skin metabolism on the effects of topically applied drugs and the factors capable of

modulating skin biotransformations are discussed. The main difficulties faced by investigators of skin metabolism stem from the relatively complex structure of skin and in the low levels of enzymatic activities.

82
West MR, Page JM, Turner DM, Wood EJ, Holland DB, Cunliffe WJ, Rupniak HT. SIMPLE ASSAYS OF RETINOID ACTIVITY AS POTENTIAL SCREENS FOR COMPOUNDS THAT MAY BE USEFUL IN TREATMENT OF PSORIASIS. J Invest Dermatol 1992; 99(1):95-100.

Human epidermal cell cultures were used to study the effects of retinoids on keratinocyte differentiation. Keratin profiles were studied by quantitative gel electrophoresis of culture extracts, whereas the extent of envelope formation was assessed in an ELISA using an antibody that specifically recognizes keratinocyte envelopes. Exposure of cultures to a variety of different retinoids produced both dose-dependent decreases in keratin 16 with consequent increases in the keratin 14:keratin 16 ratio, and a decrease in envelope formation. Analysis of the lesional keratins of psoriasis patients showed that etretinate causes a reduction in keratin 16 and an increased in the keratin 14:keratin 16 ratio, although the magnitude of these changes and their correlation with clinical improvement was variable. As the in vitro assays reported here are simple and quick, they allow rapid screening of compounds for retinoid-like activity.

83
Dick IP, Scott RC. THE INFLUENCE OF DIFFERENT STRAINS AND AGE ON IN VITRO RAT SKIN PERMEABILITY TO WATER AND MANNITOL. Pharm Res 1992;9(7):884-7.

Water and mannitol were used as test penetrants to study the effect of age on the skin permeability of the Wistar-derived Alderley Park (AP) rat and Sprague-Dawley (SD) rat. Whole-skin membranes were prepared from rats aged 10 to 120 days, while epidermal membranes were prepared from rats aged 24 to 32 days. The results indicated that the skin permeabilities of the two strains were very similar for either whole-skin or epidermal membranes. The influence of age on skin permeability was found to be negligible for the AP rat, and a small decrease in whole-skin permeability was observed for SD rats above 80 days of age. A statistically derived expression ("the sepn. efficiency

factor") was used to determine the optimum age for prepg. intact epidermal membranes. Dermal thickness, hair follicle depth, and, to a lesser extent, the surface area occupied by hair follicles all appeared to be influenced by age, although these changes had no detectable effect on skin permeability.

84
Morimoto Y, Hatanaka T, Oguchi M, Sugibayashi K, Kobayashi M, Kimura M. A SCREENING METHOD FOR PERCUTANEOUS ABSORPTION ENHANCERS APPRO-PRIATE FOR ADHESIVE MATRIX DEVICES. S.T.P. Pharma Sci. 1992; 2(3):253-8.

Permeation of a model drug, isosorbide dinitrate, through hairless rat and human skin for 6 kinds of formulation (adhesive matrix device, aqueous suspension and silicone suspensions, with or without several enhancers) was evaluated in vitro. The enhancing effect of iso-Pr myristate on the isosorbide dinitrate permeation from the adhesive matrix devices was weak compared with that from the aqueous suspension, even though the iso-Pr myristate content was the same. The efficacy of several enhancers at the same concentration was then examined in water and silicone fluids in order to estimate simultaneously the maximum enhancing potential of the enhancers and their activity in the adhesive matrix devices. Lauryl lactate and lauric acid provided a relatively high enhancing effect in both vehicles, and isosorbide dinitrate permeability from the adhesive matrix devices containing these enhancers was a few times higher than that of a commercial product, Frandol Tape S. in humans.

85
Gardner RS, Walker M, Edwardson PA D. DEVELOPMENT OF SENSITIVE HPLC ASSAYS FOR THE ANALYSIS OF STEROIDS IN IN-VITRO AND IN-VIVO STUDIES. Recent Dev Ther Drug Monit Clin Toxicol 1992;503-11.

HPLC assays were successfully developed for the detn. of very low (ng/L) concentration of corticosteroids in skin permeation (in-vitro) and tape-strip (in-vivo) investigations. The methods showed good linearity, specificity, accuracy, and precision. Sample preparation was minimal, thereby reducing cumulative errors that might otherwise occur with multistep sample preparation, as well as saving time. Analysis of very low concentrations of corticosteroids was made possible

by the use of a sensitive UV detector in connection with narrow-bore (2-mm) anal. columns. The relative ease of the methods has paved the way for a wide range of studies to be carried out on factors influencing topical drug delivery, which should be transferable to other classes of drugs.

86
Maeda K, Fukuda M. IN VITRO EFFECTIVENESS OF SEVERAL WHITENING COSMETIC COMPONENTS IN HUMAN MELANOCYTES. J Soc Cosmet Chem 1991;42(6):361-8.

The inhibitory action of arbutin, kojic acid, and ascorbic acid on tyrosinase activity in human melanocytes was compared. The substances are active whitening cosmetic components. Hydroquinone was used as a positive control. The depigmenting effect of linoleicacid, which has been reported to inhibit melanin synthesis, was compared with those of arbutin, kojic acid, and ascorbic acid. Human melanocytes were cultured with each agent in multiwell plates for three days, and the tyrosine activity was assayed using L-DOPA as a substrate. Arbutin dose-dependently reduced tyrosinase activity at final concentrations between 0.01 mM and 1.0 mM, at which no change in cell viability was seen. This action was about 1/100 that of hydroquinone, and was stronger than that of kojic acid and ascorbic acid. Linoleic acid did not reduce tyrosinase activity at non-cytotoxic ranges. At concentrations of 0.5 mM, the amount of melanin was reduced significantly by arbutin.

87
Ng KM, Chu I, Bronaugh RL, Franklin CA, Somers DA. PERCUTANEOUS ABSORPTION AND METABOLISM OF PYRENE, BENZO[A]PYRENE, AND DI(2-ETHYLHEXYL) PHTHALATE: COMPARISON OF IN VITRO AND IN VIVO RESULTS IN THE HAIRLESS GUINEA PIG. Toxicol Appl Pharmacol 1992; 115(2):216-23.

The in vitro and in vivo absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate (DEHP) were investigated in the hairless guinea pig. The in vitro method, which involved the use of flow-through diffusion cells and Hepes-buffered Hanks' balanced salt solution containing 4% bovine serum albumin as perfusate, was demonstrated to be a suitable system for predicting in vivo absorption of the above lipophilic compounds. The successful application of

the in vitro technique for these compounds is

significant because no satisfactory in vitro method has hitherto been developed to predict in vivo absorption of highly lipophilic chemicals. Quantification of parent compounds and metabolites that permeated into perfusates and those that remained in skin discs provided insight into the process by which the chemicals penetrated through the skin. Data from the present study led to the conclusion that the in vitro method can be utilized to predict in vivo absorption for compounds of high lipophilicity and that dermal metabolism facilitates partitioning of metabolites into the receptor fluid and hence may affect the biological activities of dermally applied compounds.

88
Eagle SC, Barry BW, Scott RC. DIFFERENTIAL SCANNING CALORIMETRY AND PERMEATION STUDIES TO EXAMINE SURFACTANT DAMAGE TO HUMAN SKIN. J Toxicol Cutaneous Ocul Toxicol 1992;11(1):77-92. (20 REFS)

The protective action of polyoxyethylate nonionic surfactants on the disruption of human skin by divalent anionic surfactants was studied. Abdominal skin samples obtained from human cadavers were mounted in diffusion cells. The permeability coefficient of tritiated water was measured before and after the application of surfactants. Differential scanning calorimetric (DSC) experiments were conducted to study the thermal behavior of treated skin samples. After 12 hours of contact with divalent anionic surfactants, the membrane permeability to water was increased; application of nonionic surfactants produced few or no permeability changes. When mixtures of anionic and nonionic surfactants were applied to the skin for 12 hours, each nonionic surfactant tested sufficiently protected the skin from the divalent anionic surfactants. Protection increased with numbers of ethylene-oxide units on the nonionic surfactant molecule. Four endotherms were seen in DSC experiments and each was discussed in detail. The results of the thermal studies indicated that anionic disruption of intercellular lipid or keratin within the membrane is inhibited by application of equimolar amounts of divalent anionic and nonionic surfactants.

89
Wasmus G, Bruckert HJ, Schreiber G CN. DEVELOPMENT AND

TESTING OF A METHOD FOR THE EVALUATION OF DERMAL EXPOSURE. Wirtschaftsverlag NW, Verlag fur neue Wissenschaften GmbH, Postfach 10 11 10, 2850 Bremerhaven 1, Germany, 1992. 65p. Illus. (12 REFS)

In-vivo experiments to estimate the degree to which substances can enter the body through the skin have many drawbacks. Even the acquisition of adequate samples of human skin for in-vitro measurements is difficult. Tests were conducted on a simple test chamber in which two compartments are separated by a sample of skin or polymer film. Solutions or vapors of substances to be tested were pumped through one compartment. The time until the substance could be detected on the other side and the rate of increase in its concentration were measured. The rates at which substances penetrated pig skin and polyundecanamide correlated well with the penetration rates for human skin. Thus, these two materials in the given apparatus provide a suitable system for acquiring the necessary data for establishing dermal exposure limits.

90
Richard MJ, Guiraud P, Monjo AM, Favier A. DEVELOPMENT OF A SIMPLE ANTIOXIDANT SCREENING ASSAY USING HUMAN SKIN FIBROBLASTS. Free Radic Res Commun 1992;16(5):303-14.

No abstract.

91
Darmer KI Jr, DiGiovanni J, Stevenson DE, Gill RD, Ewing MW. EVALUATION OF SUSTAINED HYPERPLASIA AND OTHER SHORT-TERM TESTS AS PREDICTORS OF TUMORIGENIC POTENTIAL IN OIL PRODUCTS. Prog Clin Biol Res 1992;374:351-66.

Some oil products are known to cause skin tumors following long-term application while others do not. The ability to predict which ones might cause tumors is important. Development of reliable short term tests which can accurately predict tumorigenic potential of oil products is needed to avoid the high cost and long time required for traditional animal bioassays. Several short term tests were evaluated for their ability to predict tumorigenic potential of 10 coded oil samples and results were compared to results of mouse bioassays. Tests which showed good correlation with bioassay results and thus were considered good predictors of tumorigenic potential were: Sustained Epidermal Hyperplasia as measured by epidermal

thickness, Nuclear Area of epidermal basal cells, Modified Ames Test and DMSO extraction for PAC content. Tests which did not show good correlation with bioassay results and which were not considered good predictors of tumorigenic potential were: Polymorphonuclear leukocyte (PMN) infiltration into the dermis, unscheduled DNA synthesis in epidermal cells and changes in nuclear DNA content of CHO cells.

92
van Erp YH, Koopmans MJ, Heirbaut PR, van der Hoeven JC, Weterings PJ. UNSCHEDULED DNA SYNTHESIS IN HUMAN HAIR FOLLICLES AFTER IN VITRO EXPOSURE TO 11 CHEMICALS: COMPARISON WITH UNSCHEDULED DNA SYNTHESIS IN RAT HEPATOCYTES. Mutat Res 1992;271(3):201-8.

A new method is described to investigate unscheduled DNA synthesis (UDS) in human tissue after exposure in vitro: the human hair follicle. A histological technique was applied to assess cytotoxicity and UDS in the same hair follicle cells. UDS induction was examined for 11 chemicals and the results were compared with literature findings for UDS in rat hepatocytes. Most chemicals inducing UDS in rat hepatocytes raised DNA repair at comparable concentrations in the hair follicle. However, 1 of 9 chemicals that gave a positive response in the rat hepatocyte UDS test, 2-acetylaminofluorene, failed to induce DNA repair in the hair follicle. Metabolizing potential of hair follicle cells was shown in experiments with indirectly acting compounds, i.e., benzo[a]pyrene, 7,12-dimethylbenz[a]anthracene and dimethylnitrosamine. The results support the conclusion that the test in its present state is valuable as a screening assay for the detection of unscheduled DNA synthesis. Moreover, the use of human tissues may result in a better extrapolation to man.

TERATOGENICITY
93
Hales BF. TERATOGENICITY. In Vitro Toxic. Test. 1992:205-20. (39 REF)

No abstract.

94
Nito S, Ariyuki F, Nakayama Y. A NEW IN VITRO SCREENING METHOD FOR TERATOGENS USING HUMAN EMBRYONIC PALATAL

MESENCHYMAL CELLS. Congenital Anom 1991;31(4):329-36.

In order to establish an in vitro screening assay system for cleft palate-inducing teratogens, 31 teratogenic and 10 nonteratogenic compounds were tested using human embryonic cultured cells. The authors examined whether cleft palate-inducing ability can be detected by differential growth inhibition between human embryonic palatal mesenchymal (HEPM) cells and human embryonic fibroblasts (MRC-5). Thirty one compounds with proven cleft palate-inductive effects in vivo preferentially inhibited the proliferation of HEPM cells. The average of the relative inhibitory rates (rate of IC50 value for HEPM cells to MRC-5 cells) of teratogens was 0.53. In contrast, almost all nonteratogens identically inhibited the proliferation of both cell lines and the average of the relative resistant rates was 1.01. The results indicate that teratogens which induce cleft palate in vivo preferentially inhibit the proliferation of embryonic palatal mesenchymal cells. The data indicated that in vitro screening using HEPM and MRC-5 cells is useful for detecting the cleft palate-inducing ability of chems.

95
Hales BF. IN VITRO APPROACHES TO TERATOLOGY. Altern Methods Toxicol 1991; 8 Iss In Vitro Toxicol: Mech New Technol:77-86. (36 REFS)

Teratogenesis in vitro assay review.

TISSUE CULTURE
96
Krall JF. ENHANCING SMOOTH MUSCLE PROPERTIES OF CULTURED RAT MYOMETRIUM PNEUMONIA. Veterans Administration/Research and Development (15), 810 Vermont Ave. N.W., Washington, D.C. 20420, United States of America. Fedrip Database, National Technical Information Service (NTIS), Springfield, VA, USA.

Because of the dangers to both mother and fetus, evaluation of hazardous chemicals that contribute to premature labor or spontaneous abortion by the effects they have on uterine motility requires non-human mammalian models. The purpose of this project was to reduce reliance on intact animal testing by developing a useful tissue culture, i.e. in vitro, alternative for evaluating the toxicity of ethanol, a drug which is

clinically useful in delaying premature labor, yet hazardous to the fetus. RESEARCH PLAN: Uterine motility resides within the smooth muscle layers of the uterus (myometrium). Smooth muscle cells are isolated from the myometrium of the 21 day old rat and perpetuated in tissue culture as a renewable resource on which to test ethanol as well as its principal metabolite, acetaldehyde. METHODS: In the intact animal, smooth muscle cell contraction and relaxation is correlated most closely with intracellular calcium, and the study of the control of uterine motility is, therefore, the study of the regulation of intracellular calcium. How exposure to ethanol or acetaldehyde affects intracellular calcium changes caused by hormones or neurotransmitters that are known to affect uterine motility is characterized in the cultured smooth muscle cells. CONCLUSIONS: There were dramatic differences in intracellular calcium, inositol phosphate production, and in ethanol sensitivity between growing cultures and cultures that have had their growth arrested. Results thus far indicate that either growing or non-growing smooth muscle cultures, but not both, may be a suitable myometrial model for in vitro toxicity testing.

TOXICITY (GENERAL)
97
Parascandola J. HISTORICAL PERSPECTIVES ON IN VITRO TOXICOLOGY. Altern Methods Toxicol 1991; 8, ISS In Vitro Toxicol: Mech New Technol:87-96. (24 REFS)

The use of in vitro toxicity tests to determine the safety of food additives, drugs, cosmetics and other substances is a relatively recent development, but toxicological testing itself dates back to early times. Pharmacology and toxicology did not emerge as systematic sciences, however, until the nineteenth century, and from their very beginnings were confronted by opposition to vivisection experiments. Animal experimentation came to occupy an increasingly important role in medical research in the twentieth century. The number of new chemical substances (drugs, insecticides, food additives, etc.) entering the marketplace also rose dramatically as the twentieth century progressed, and concerns about the safety of these products grew accordingly. As public pressures to protect the consumer heightened, legislation in the 1950s and 1960s introduced or strengthened regulations concerning the premarket toxicity testing of food additives, color additives, insecticides, drugs and other products. Concerns about toxins in the

environment and in the workplace led to further legislation regarding the testing and control of toxic substances. At the same time, the animal protection movement was undergoing a revival in the United States. All of these factors have contributed to an increased in the concept of alternatives to the use of animals in toxicological testing. There was some use of tissue culture techniques to measure toxicity at least as far back as the early twentieth century, but the first real call for a specific goal of developing alternatives to animal studies came from British scientist W. M. S. Russell in the late 1950s. Stimulated by pressure from animal rights activists, as well as by economic considerations and scientific developments, efforts to develop in vitro toxicity tests have greatly intensified in the past twenty-five years.

VALIDATION TESTS

98

Claxton LD, Douglas G, Krewski D, Lewtas J, Matsushita H, Rosenkranz H. OVERVIEW, CONCLUSIONS, AND RECOMMENDATIONS OF THE IPCS COLLABORATIVE STUDY ON COMPLEX MIXTURES. Mutation Research 1992; 276(1/2):61-80. (32 REFS)

An overview of the findings and conclusions of a collaborative study sponsored by the International Programme on Chemical Safety (IPCS) on the mutagenicity of complex mixtures was presented. Twenty laboratories participated. Topics included guiding concepts and information, sources of variation, standard reference material (SRM) mutagenicity reference values, use of other bioassays, characterization of genotoxic components in chemical mixtures, and the potential for future collaborative efforts. Results indicated a need to: design factorial experiments to determine the contribution of specific protocol components to overall variance; establish marker compounds and terminology for the SRMs best able to define chemical classes; establish a central laboratory to examine the kinetics associated with the storage of environmental samples and SRMs; perform studies using these same SRMs to establish a detailed protocol; direct research efforts toward normalization of bioassay results; use new batches of the SRMs to develop water related SRMs and other types of SRMs; and, to evaluate other short term in-vitro assays.

XYZ/MISCELLANEOUS

99
Hitchins VM, Lytle CD, Withrow TJ, Thomas DP, Sigler CI, Stahl RW, Harbell JW. RESPONSE OF RAW 264.7 MURINE MACROPHAGE CULTURED CELLS TO UVC RADIATION. In Vitro Toxicol 1992;5(1):39-49.

No abstract.

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This paper reviews the development of the polyanhydrides as bioerodible polymers for drug delivery applications. The topics include design and synthesis of the polymer, physical properties, techniques to fabricate the polymer into drug delivery devices, evaluation of biocompatibility, and example applications of the polyanhydrides. Discussion of the interrelationship between the physical-chemical properties of the polyanhydrides, fabrication methods, and drug release rates is included. One section includes an outline of the extensive in vitro and in vivo testing that is necessary for development of a new material for biomedical applications.