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

A Bibliography with Abstracts

To Assist In:

- Refining Existing Test Methods
- Reducing Animal Usage
- Replacing Animals As Test Systems

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

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

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Suggestions and comments are welcome.

BRAIN/CNS

1

Stehrer-Schmid P, Wolf HU. THE IN-VITRO PORCINE BRAIN TUBULIN ASSEMBLY ASSAY EFFECT OF FOUR CARBAMATE INSECTICIDES AND DEVELOPMENT OF A MODIFIED EVALUATION PROCEDURE. 35th Spring Meeting of the German Society for Experimental and Clinical Pharmacology and Toxicology, Mainz, Germany, March 15-17, 1994. Naunyn-Schmiedeberg's Archives of Pharmacology 1994; 349(Suppl):R129.

No abstract.

2

Gupta RP, Abou-Donia MB. IN-VIVO AND IN-VITRO EFFECTS OF DIISOPROPYL PHOSPHOROFLUORIDATE (DFP) ON THE RATE OF HEN BRAIN TUBULIN POLYMERIZATION. Neurochemical Research 1994; 19(4):435-444.

Diisopropyl phosphorofluoridate (DFP) produces organophosphorus ester-induced delayed neurotoxicity (OPIDN) in sensitive species. We have investigated the in vivo and in vitro effects of DFP on hen brain tubulin polymerization. Hens were treated with a single dose of DFP (1.7 mg/kg, sc.), and were sacrificed after 18-21 days. Tubulin from DFP-treated hen brains showed small but significant decrease (14.42%) in the rate of polymerization and 11.05% decrease in rise in O.D. at 340 nm in 30 min. DFP in vivo treatment also resulted in decreased concentration of tau and an enhanced concentration of two peptides (45 kDa, 35 kDa) in the brain supernatant. These peptides seemed to be the degradation products of MAP-2. The decrease in the rate of brain tubulin polymerization in treated hens is consistent with neurochemical alterations and the focal degeneration and aggregation of these filamentous structures in OPIDN.

3

Dehouck M, Jolliet-Riant P, Bree F, Tillement J, Dehouck B, Fruchart J, Cecchelli R. IN VITRO RECONSTITUTED BLOOD-BRAIN BARRIER: A METHOD TO SCREEN NEW CENTRALLY ACTING DRUGS. Biol Prospect 1993, C. R. Colloq. Pont-a-Mousson, 8th:463-6.

The strong correlation between the "in vivo" brain extn. and in vitro passage extn. values demonstrated that this in vitro model will bean important tool for investigations of the role of the BBB in the delivery of drugs to the brain. Furthermore, the relative ease with which such cocultures can be produced in large quantities and the reproducibility of the system would facilitate not only the study of the biochem. mechanisms regulating peptide delivery to the central nervous system at the cellular level, but also provide a more efficient and selective system for the

screening of new centrally active peptides.

CANCER/TUMOR TOXICITY
4
Brandes LJ, Warrington RC, Aron RJ, Bogdanovic RP, Fang W, Queen GM, Stein DA, Tong J, Zaborniak C LF, Labella FS. ENHANCED CANCER GROWTH IN MICE ADMINISTERED DAILY HUMAN-EQUIVALENT DOSES OF SOME H1-ANTIHISTAMINES: PREDICTIVE IN VITRO CORRELATES.
J National Cancer Institute (Bethesda) 1994;86(10):770-775.

Background: Present studies of drug-induced tumor growth promotion have evolved from earlier investigations into the mechanism of action of N,N-diethyl-2-(4-(phenylmethyl)phenoxy)ethanamineich potently inhibits lymphocyte mitogenesis in vitro and stimulates tumor growth in vivo. It is thought that potency to bind to intracellular histamine receptors (HIC), some of which are on cytochromes P450, may correlate with tumor growth-promoting activity. Purpose: We assessed the effectiveness of five in vitro assays in predicting in vivo tumor growth stimulation by the H1-antihistamines loratadine, astemizole, cetirizine, bydroxyzine, and doxylamine. Methods: Potency of each agent was ranked 1-5 in each of the following in vitro assays: 1) inhibition of (3H)histamine binding to microsomal HIC, 2) inhibition of histamine binding to microsomal P450, 3) inhibition of the P450-catalyzed demethylation of aminopyrine, 4) inhibition of lymphocyte mitogenesis, and 5) stimulation of tumor colony formation. An overall rank score was assigned to each drug and correlated with tumor growth stimulation in vivo. Two laboratories conducted in vivo studies in a blinded fashion. Female C57BL and C3H mice were given a subcutaneous injection on day 1 of syngeneic B16F10 melanoma cells (5), respectively. Mice were randomly assigned to treatment groups, then received a single, daily intraperitoneal injection of an estimated human-equivalent dose (or range of doses) of antihistamine or vehicle control for 18-21 days before being killed. Tumors were surgically removed and wet weights compared statistically among groups. Results: The cumulative potency of each drug in affecting tumor growth or growth mechanisms in the rive in vitro assays ranked as follows: Loratidine and astemizole ranked highest and

were equally potent, followed in decreasing order by hydroxyzine, doxylamine, and cetirizine. A significant correlation ($r = .97$; $P<.02$) was observed between the rank order of potency of the antihistamines in all five in vitro assays and the rank order to enhance tumor growth in vivo: Loratidine and astemizole significantly ($P<.001$) promoted the growth of both melanoma and fibrosarcoma, hydroxyzine significantly ($P<.001$) promoted the growth of melanoma, while doxylamine and cetirizine did not promote the growth of either tumor. Conclusion: Data demonstrate

that the in vitro assays predicted the propensity of each H1-antihistamine to stimulate cancer growth in vivo. Implication: These in vitro tests may prove valuable to screen potential tumor growth promoters.

CELL CULTURE

5

Flaskos J, Mclean WG, Hargreaves AJ. THE TOXICITY OF ORGANOPHOSPHATE COMPOUNDS TOWARDS CULTURED PC12 CELLS. Toxicol Lett (Shannon) 1994;70(1):71-76.

The effects of three representative organophosphates (OPs), tricresyl phosphate (TCP), triphenyl phosphite (TPP) and paraoxon (POX) on the proliferation and viability of rat PC12 pheochromocytoma cells were studied. With respect to its IC50, TCP was at least an order of magnitude more potent in its antiproliferative activity than both TPP and POX. All test OPs were cytotoxic at concentrations inhibiting cell proliferation. No compound inhibited cell growth below 10 mug/ml. For TCP and TPP the estimated IC50 values from proliferation assays were lower than published LD50 values in vivo, whereas paraoxon was much less toxic in vitro than in vivo. Subcytotoxic levels of TCP (1 mug/ml) were found to inhibit the maintenance of neurites on cells grown in the presence of nerve growth factor.

6

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

An in vitro cell culture assay using myeloma cells and hybrid lymphocytes was developed which detected pathogenic Listeria strains in just 6 h. Three separate hybridoma cell lines, murine Ped-2E9 and EM-7G1 and human RI.37 and murine myeloma NS1 cells, proved equally sensitive in responding to virulent Listeria species. Listeria monocytogenes along with other Listeria spp., collected from food and clinical sources, were inoculated at 108 cfu/ml into a suspension of Ped-2E9 (106/ml). Pathogenic Listeria spp. killed 80% of hybridoma cells by 4 h, as determined by trypan blue exclusion test. Conversely, none of all nonpathogenic Listeria spp. killed the hybridoma cells. Ped-2E9 cells exposed to three strains of L. monocytogenes strains showed 96-97.5% death in 6 h measured by trypan blue staining and release of 91-97% of lactate dehydrogenase (LDH) enzyme. RI.37 cells showed similar results. A multiplicity of exposure (MOE) of 100 L. monocytogenes to 1 hybridoma cell or of 10:1 killed about 80% of the hybridoma cells in 4 or 6 h respectively. The in vitro virulence assay of L. monocytogenes with hybridoma cells compared

favorably with the immunocompromised mouse model, yielding results in 6 h instead of 3 days. Intracellular L. monocytogenes and L. innocua were not recovered from Ped-2E9 hybridoma cells after 2 or 4 h of exposure. However, attachment of both L. monocytogenes and L. innocua cells on Ped-2E9 cell surfaces were observed under epifluorescence microscopy. Direct contact of hemolysin positive L. monocytogenes with hybridoma cells is essential to cause death, since hybridoma cells were not killed when they were separated from the growing bacteria by a 0.45 mum filter.

7
Guelden M, Seibert H, Voss J-U. IN VITRO TOXICITY SCREENING USING CULTURED RAT SKELETAL MUSCLE CELLS. II. AGENTS AFFECTING EXCITABLE MEMBRANES. Toxicol In Vitro 1994;8(2):197-206.

The screening of chemicals for their potential to interfere with excitable cell membranes should be an important element of in vitro testing for acute toxicity. The suitability for this purpose of a test system using primary cultured rat skeletal muscle cells was evaluated. The test protocol involved the determination of the concentration-dependent effects on three endpoints: (1) spontaneous contractility, (2) membrane integrity and (3) energy metabolism. The chemicals investigated were: NaCl, KCl and CaCl2; cardiac glycosides (ouabain, digoxin); sodium channel toxins (tetrodotoxin, saxitoxin, veratridine, Anemonia sulcata toxin II, Bolocera tuediae toxin II); an acetylcholine agonist (carbachol); a calcium antagonist (D600) and three membrane-directed insecticides (deltamethrin, DDT, lindane). The response pattern of most of these substances-alteration of contractility at concentrations that neither affected the energy metabolism nor were cytolethal-characterized them as acting either on the excitable membrane or on the excitation-contraction coupling and the contractile apparatus. The results indicate that the test system is suited to assess chemical effects resulting in: (i) changes of resting membrane and threshold potentials, (ii) altered sodium channel function, (iii) opening of endplate channels, (iv) blockade of calcium channels, and (v) inhibition of Na+/K+-ATPase.

8
Kohler M, Aufderheide M, Ramm D. METHOD FOR THE DESCRIPTION OF DIFFERENCES IN THE FILAMENTOUS STRUCTURE OF THE CYTOSKELETON IN CULTURED CELLS. Toxicol Lett 1994;72(1-3):33-42.

Over the last few years in vitro cell systems have been established for toxicological investigations. These systems permit the evaluation of effects on the basis of cultured cells in order to replace animal studies. Not only qualitative

assessment of cytotoxic effects, but also efforts to quantify these intracellular changes have become more and more important to objectify the results which have been obtained. The cytoskeleton, a dynamic and sensitive system, seems to be a valuable morphological parameter to gain information about the intracellular alterations of drug-influenced cells. Depending on the dose of the substance administered, the cytoskeleton shows morphological alterations in specific components, which fulfill structural as well as metabolic regulatory functions and thus provide information on possible mechanisms. Normally, microtubules as well as the intermediate filament system from 3-dimensional networks. Treatment may induce contraction or depolymerization of the filamentous proteins. These alterations, seen in immunofluorescent preparations, can be quantified by means of a 2-dimensional Fourier transformation. As there is no statistical method to compare different spectra, the spatial frequency spectrum of the Fourier components has to be transformed to a 1-dimensional array. This step is performed by measuring the optical density of localised areas in the frequency spectrum. Using this transformation it is possible to compare the Fourier spectra belonging to different treatment groups.

9
Tsutsiu T, Barrett JC. MULTISTEP MECHANISM FOR IMMORTALIZATION OF HUMAN FIBROBLASTS BY CARCINOGENS. 85th Annual Meeting of the American Association for Cancer Research, San Francisco, California, USA, April 10-13, 1994. Proceedings of the American Association for Cancer Research Annual Meeting 1994;35(0):126.

No abstract.

10
Acheson D, Jacewicz M, Skutelsky E, Keusch GT, Moore R. DEVELOPMENT OF AN IN VITRO MODEL USING CULTURED GASTROINTESTINAL CELL LINES FOR STUDYING TRANSLOCATION OF SHIGA-LIKE TOXINS. Gastroenterology 1994;106(4 Suppl):A642.

No abstract.

11
Qiu Q, Vincent P, Lowenberg B, Sayer M, Davies JE. BONE GROWTH ON SOL-GEL CALCIUM PHOSPHATE THIN FILMS IN VITRO. Cells Mater 1993;3(4):351-60.

Thin, sub-micron, films of calcium phosphate were fabricated on either glass or quartz supports by a colloidal suspension sol-gel method. These films, which varied in both surface chemicals and topog. were then employed as culture substrata for osteogenic rat

bone marrow cells. During an 18-day culture period, the cells elaborated a morphol. distinguishable bone matrix on all substrata which was similar to that reported earlier on tissue culture polystyrene. Selected samples of the culture substrata were fractured, crit. point dried, and observed by SEM. Particular attention was paid to the morphologies of the interface between the sol-gel layer and the underlying support, and that between the sol-gel layer and the elaborated bone tissue. The mech. disruption of both tissue and thin films resulting from crit. point drying affected the morphol. of both interfaces dependent upon the film processing conditions. The interfacial bone matrix, which was a cement-line like matrix, interdigited with the surface of the films. This mech. interdigitation created a bond which remained intact during tissue processing. With films processed at 1000.degree. on quartz supports, but not with those processed at lower temps. on glass, fracture of the interface revealed pitting in the quartz surface which was assocd. with areas of adherence of the overlying calcium phosphate film. These preliminary studies demonstrate the intimate relation which can be established between such thin calcium phosphate thin films and bone matrix.

12
Bradlaw J, Pritchard D, Flynn T, Eppley R, Stack M. IN VITRO ASSESSMENT OF FUMONISIN B-1 TOXICITY USING REAGGREGATE CULTURES OF CHICK EMBRYO NEURAL RETINA CELLS CERC. Meeting of the Tissue Culture Association on Regulation of Cell and Tissue Differentiation, Research Triangle Park, North Carolina, USA, June 4-7, 1994. In Vitro Cellular & Developmental Biology Animal 1994;30A(3 Part 2):92.

No abstract.

13
Pritchard D, Obermeyer W, Bradlaw J, Roth W, Flynn T, Yates J, Page S. PRIMARY RAT HEPATOCYTE CULTURES AID IN THE CHEMICAL IDENTIFICATION OF TOXIC CHAPARRAL LARREA TRIDENTATA FRACTIONS. Meeting of the Tissue Culture Association on Regulation of Cell and Tissue Differentiation, Research Triangle Park, North Carolina, USA, June 4-7, 1994. In vitro Cellular & Developmental Biology Animal 1994;30A(3 Part 2):91-92.

No abstract.

14
Liu HS, Biing JT, Yang YF, Chao CF. ESTABLISHMENT AND CHARACTERIZATION OF THE TRANSFECTABLE GOLDEN HAMSTER EMBRYO FIBROBLAST CELL LINE. Proc Natl Sci Counc Repub China B

1994;18(1):1-11.

A diploid, continuous cell line, Golden Hamster Embryo Fibroblast-III (GHEF-III), which had been passaged for one year, was established essentially by a 3T3 protocol from primary culture of 14-day-gestation Golden Hamster embryo fibroblast cells. The cultured fibroblast exhibited monolayer growth and had contact inhibition. In morphological identification by light microscope (LM), transmission electron microscope (TEM) and immunofluorescence examination (IF), these multipolar and

spindle-shaped cells had a large ovoid nucleus, enriched rER and mitochondria in the cytoplasm. On the other hand, the vimentin presented in the cell with a random network and capped around the nucleus. The results indicated that the cultured GHEF-III cells were fibroblast in origin. The cells were free of bacterial and mycoplasma contamination. The doubling time in GHEF-III was about 15 hours. Chromosomal analysis of GHEF-III presented a diploid stem cell line with a modal number of 44. No evidence of transformation of GHEF-III was shown by properties of contact inhibition, no colony formation in soft agar, and no tumor growth in nude mice. The transformation of GHEF-III after transfection with pT24-C3, an oncogenic plasmid, was shown by the evidence of loss of contact inhibition, growth in low serum medium, colony formation in soft agar and tumor growth in nude mice. In vitro transformation testing of these cells may provide valuable data in studying the role of tumor transforming genes in carcinogenesis. Owing to the genetic stability and less spontaneous transformation, this GHEF-III cell line can be utilized as a source of recipient cells in transfection assay.

CYTOTOXICITY

15

Clemedson C, Abdulla E, Barile FA, Chesne C, Clothier R, Cottin M, Curren R, Dierickx P, Ferro M, Et al. COMPARISON OF THE TOXICITY OF 30 CHEMICALS AS MEASURED BY 68 DIFFERENT IN VITRO TOXICITY TESTS. Meeting of the Tissue Culture Association on Regulation of Cell and Tissue Differentiation, Research Triangle Park, North Carolina, USA, June 4-7, 1994. In Vitro Cellular & Developmental Biology Animal 1994;30A(3 Part 2):90-91.

No abstract.

16

Barile FA, Alexander D. IN VITRO CYTOTOXICITY TESTING 72-HOUR STUDIES WITH CULTURED LUNG CELLS. Meeting of the Tissue Culture Association on Regulation of Cell and Tissue Differentiation, Research Triangle Park, North Carolina, USA, June 4-7, 1994. In Vitro Cellular & Developmental Biology Animal 1994;30A(3 Part

2):90.

No abstract.

17
Hamberger JF, Peters CJ, Jessee CB. QUALITY CONTROL OF L929 CELLS FOR USE IN IN VITRO CYTOTOXICITY METHODS A TEST BATTERY TO REVEAL PASSAGE-DEPENDENT ALTERATIONS IN CELLULAR RESPONSES. Meeting of the Tissue Culture Association on Regulation of Cell and Tissue Differentiation, Research Triangle Park, North Carolina, USA, June 4-7, 1994. In Vitro Cellular & Developmental Biology Animal 1994;30A(3 Part 2):88.

No abstract.

18
Juneja C, Stott CW. DELAYED EXPRESSION OF CYTOTOXICITY IN NORMAL HUMAN EPIDERMAL KERATINOCYTES TREATED WITH SHAMPOOS. Meeting of the Tissue Culture Association on Regulation of Cell and Tissue Differentiation, Research Triangle Park, North Carolina, USA, June 4-7, 1994. In Vitro Cellular & Developmental Biology Animal 1994;30A(3 Part 2):85.

No abstract.

19
Boyce ST, Sheeler CQ, Holder IA. ASSAY OF CYTOTOXICITY OF ANTIMICROBIAL AGENTS BY CORRELATION OF KERATINOCYTE NUMBERS WITH OPTICAL DENSITY OF CRYSTAL VIOLET. Meeting of the Tissue Culture Association on Regulation of Cell and Tissue Differentiation, Research Triangle Park, North Carolina, USA, June 4-7, 1994. In Vitro Cellular & Developmental Biology Animal 1994;30A(3 Part 2):84-85.

No abstract.

20
Korting HC, Herzinger T, Hartinger A, Kerscher M, Angerpointner T, Maibach HI. DISCRIMINATION OF THE IRRITANCY POTENTIAL OF SURFACTANTS IN VITRO BY TWO CYTOTOXICITY ASSAYS USING NORMAL HUMAN KERATINOCYTES, HaCaT CELLS AND 3T3 MOUSE FIBROBLASTS: CORRELATION WITH IN VIVO DATA FROM A SOAP CHAMBER ASSAY. J Dermatol Sci 1994;7(2):119-129.

Cell cultures have been proposed as a promising model for local tolerance testing. This study evaluated the cytotoxic effects of surfactants on early passage normal human keratinocytes,

transformed human keratinocytes (HaCaT cells) and Swiss 3T3 embryonic mouse fibroblasts. Cell membrane integrity, as assessed by the release of the vital dye neutral red, and cell proliferation, as assessed by measurement of the total protein content, were both affected in a dose-dependent manner in response to surfactant exposure. There was a close correlation between the dose-response characteristics for the three cell types. Two surfactants exhibited differential effects on membrane integrity and proliferation, and thus no significant correlation was found between the two endpoints. The irritation potential of the surfactants to human forearm skin in vivo was assessed in a soap chamber test using transepidermal water loss and skin redness as quantitative endpoints. A comparison between the responses in vivo and in vitro yielded the highest correlation for the neutral red release test on normal keratinocytes. The total protein test did not significantly correlate with the soap chamber assay for keratinocytes and HaCaT cells. These results suggest that cultured normal human keratinocytes may be predictive for the irritancy of various surfactants in man. Definite judgement, however, has still to be based on confirmation in human volunteers of larger groups of compounds with diverse physico-chemical properties.

21
Hart CA, Kathman LM, Hesterberg TW. IN VITRO CYTOTOXICITY OF ASBESTOS AND MAN-MADE VITREOUS FIBERS: ROLES OF FIBER LENGTH, DIAMETER AND COMPOSITION. Carcinogenesis (Oxford) 1994; 15(5):971-977.

The present study investigated (i) the impact of various fiber parameters on in vitro toxicity to cells and (ii) the validity of an in vitro test system as a toxic screen for fibrous materials. Chinese hamster ovary cells were exposed in vitro to a series of size-selected inorganic test fibers that represented a range of different diameters, lengths and compositions (glass, refractory ceramic, mineral wool,asbestos). Toxic end-points included inhibition of proliferation, induction of micronuclei and polynuclei and viability. For all compositions tested, toxic effects were similar: a concentration-dependent decrease in proliferation and increase in incidence of morphologically abnormal nuclei with minor decreases in viability. Diameter-dependent differences in toxicity were slight or absent for fiber diameters ranging from 0.3 - 7 mum when concentration was expressed as number of fibers/cm2. Length-dependent differences in toxicity were, however, striking. EC50 values (concentration in fibers/cm2 that reduced cell proliferation to 50% of unexposed control cultures) plotted against fiber length produced a hyperbolic curve, demonstrating that toxicity increases with fiber length up to 20 mum. All fibers tested fell on this hyperbola. These data suggest that: (a) the primary toxic

effect of fibers on CHO cells is the induction of nuclear morphologic alterations resulting in cytostasis; (b) fiber diameter has little or no impact on in vitro toxicity when concentration is calculated as fibers/cm2; (c) fiber length is directly proportional to in vitro toxicity; and (d) toxicity of asbestos and vitreous fibers to CHO cells is not affected by composition. The lack of compositional effect in CHO cells does not correlate with findings from recent rodent inhalation studies using the same test fibers. Thus CHO cells may not be an appropriate in vitro model of fiber pathogenesis and would not constitute a valid toxicologic screening system for fibers.

22
Knebel JW, Aufderheide M, Emura M. COMPARISON OF BIOLOGICAL EFFECTS OF DIFFERENT POLYCYCLIC AROMATIC HYDROCARBONS IN LUNG CELLS OF HAMSTER AND RAT IN VITRO. Toxicol Lett 1994; 72(1-3):65-72.

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

23
Rotter BA, Thompson BK, Clarkin S, Owen TC. RAPID COLORIMETRIC BIOASSAY FOR SCREENING OF FUSARIUM MYCOTOXINS. Natural Toxins 1993;1(5):303-307.

The cytotoxicity of Fusarium mycotoxins was evaluated using a trichothecene sensitive cell line (BHK-21, baby hamster kidney cells) in combination with the MTT-cleavage test as an end-point measurement. Cells tended to be more sensitive to the type A trichothecenes with midpoint cytotoxicity values ranging from 1.6 ng/ml for T-2 toxin to 60 ng/ml for scirpentriol. The cytotoxicity value for deoxynivalenol (type B) was 112 ng/ml. The

inherent disadvantage of the MTT-assay (formation of insoluble formazan) was overcome by using the analog MTS and measuring the water-soluble formazan directly in the culture media. The MTS-midpoint cytotoxicity values for T-2 toxin and deoxynivalenol (2.1 and 141 ng/ml, respectively), although slightly higher, showed a good correspondence to the MTT-test. Both the MTT- and MTS-cleavage tests are useful for evaluating the cytotoxicity of Fusarium mycotoxins. The replacement of MTT by MTS substantially reduced the number of sample processing steps and the length of time required to complete the cytotoxicity assay.

24
Abdul-Hussain S, Acosta D. IN VITRO MODEL TO EVALUATE THE CYTOTOXICITY OF TWO GROUPS OF SURFACTANT MIXTURES WITH RAT KERATINOCYTE CULTURES. Toxic Subst J 1994;13(1):1-14.

Keratinocyte cultures were isolated and established from rat (1 d old) skin and used in this study as an in vitro testing system. Because of the widespread use of surfactants in consumer products, their cytotoxicity was investigated in this in vitro study. Two groups of surfactants were used. The first group contained Miranol HM 24% (HMS; lauroamphodiacetate) and sodium dodecyl sulfate (SDS) in varying concns., and the second group had Tween-20 [polyoxyethylene (20) sorbitan monolaurate] in addn. to HMS and SDS. On d 3 after seeding, keratinocyte cultures were exposed to 5 different concns. of each combination for 1 h. Cellular toxicity was assessed by lactate dehydrogenase (LDH) leakage and by mitochondrial enzymic activity, using the 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) assay at 1 and 24 h after chem. exposure. Additional, a concn.-response curve was constructed to determine the EC50, and its value was computed at 1 h after chem. exposure. Results indicate that this in vitro system may be useful in assessing cytotoxicity potential.

25
Pizao PE, Winograd B, Peters GJ, Leyva A, Giaccone G, Pinedo HM. IN VITRO CHEMOSENSITIVITY TESTING OF MULTILAYERED MICROCULTURES. Anticancer Res 1992;12(4):1319-22.

A potential limitation of in vitro microtiter cytotoxicity assays as compared to in vivo antitumor studies is that the complex three-dimensional structure of the solid tumor is lost in monolayer cultures in vitro. The authors investigated whether more in vivo like cell-cell interactions could be easily and reproducibly obtained in an in vitro cytotoxicity assay. HT29 human colon adenocarcinoma cells were seeded in 96-well microtiter plates with << V >>-shaped wells and allowed to form postconfluent multilayered cultures. Cross-sections of

micro-cultures fixed after 2 and 3 wk following plating revealed approx. 7 and 35 cell layers, resp. Using a tetrazolium assay to assess cytotoxicity, the EC50 (drug concn. which gives absorbance readings 50% lower than those of non-treated wells) of multilayered cultures exposed to doxorubicin for 24 h was 12 times higher ($p < 0.05$) than that detd. for subconfluent monolayered cells simultaneously exposed to the drug. This system offers an alternative to study the chemosensitivity of three-dimensionally organized cells using semiautomated microtiter plate technol.

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Parker TL, Parker KL, McColl IR, Grant DM, Wood JV.
THE BIOCOMPATIBILITY OF LOW TEMPERATURE DIAMOND-LIKE CARBON FILMS: A TRANSMISSION ELECTRON MICROSCOPY, SCANNING ELECTRON MICROSCOPY AND CYTOTOXICITY STUDY. Diamond Relat Mater 1994; 3(8):1120-3.

Preliminary studies have shown that diamond-like carbon (DLC) coatings can be adherent on a range of substrate materials, resistant to wear and non-toxic to cells in vitro. This has led to the question of the possible biomedical application of DLC-coated materials, particularly in the field of implantation surgery. Assessment of the biocompatibility of implanted material requires two questions to be addressed: (1) is the implanted material toxic to cell growth, and (2) what is the nature of the cell-DLC layer interaction. In this study the authors show, by the Kenacid Blue cytotoxicity test, that cells grown on DLC-coated PCF membranes exhibit an initial increased attachment to the carbon substrate, and thereafter exhibit a normal growth rate over the 3-day test periods compared with cells grown on uncoated polystyrene. SEM shows these cells possess characteristics typical of normal cell attachment and growth. Cells were grown on DLC-coated PCF membranes to facilitate examn. of the cell-DLC coating interface by transmission electron microscopy. These results show that cells grown on both DLC-coated and non-coated membranes presented normal morphol. characteristics of attachment and growth. These findings indicate that the DLC coating is non-toxic in vitro and sustains normal cell growth.

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Sugiki Y, Yamazaki N, Matsuoka A, Suzuki T, Hayashi M, Sofuni T.
COMPARISON OF SIX CYTOTOXICITY TESTS TO FIND OPTIMAL DOSE RANGE FOR THE IN VITRO CHROMOSOMAL ABERRATION TEST. Kankyo Hen'igen Kenkyu 1994;16(1):37-43.

To find the optimal dose range of chems. for the in vitro chromosomal aberration test using Chinese hamster cells,

regulatory guidelines suggest some cytotoxic indicators, e.g., cell confluency, viable cell no., colony forming efficiency, and mitotic index. The cell growth inhibition test using "Monocellater", which measures all confluency is widely used in Japan. The authors compared six different cytotoxic indicators for dose-finding: (1) cell growth inhibition measured by Monocellater (Olympus Opt. Co., Ltd.), (2) cell growth inhibition by crystal violet staining using 96-well plates, (3) viable cell count by trypan blue staining, (4) measurement of lactate dehydrogenase (LDH) activity released from damaged cells into culture medium, (5) colony forming efficiency by direct and replating methods (CFE), and (6) mitotic index evaluation of the slides analyzed for chromosomal aberrations. A Chinese hamster lung fibroblast cell line, CHL/IU, was treated with four model chemicals (MNNG, potassium bromate, hydrogen peroxide, and cetylpyridinium chloride monohydrate) and analyzed by optimized protocols for each assay. To evaluate the relation of cytotoxic and clastogenic effect, the in vitro chromosomal aberration test was also performed using CHL/IU cells. All assays showed dose-dependent cytotoxicity, to all model chems., except for the LDH assay, which responded only to cetylpyridinium chloride monohydrate. The CFE differ from the chromosomal aberration assay in test condition, and was the most sensitive among test studied. The authors could obsd. metaphase chromosomes, even when CFE value was reduced to almost 0%. Thus the LDH assay and the CFE have limited values for does selection. The other four cytotoxic endpoints appear to be acceptable for preliminary dose finding tests in the in vitro chromosomal aberration test.

GENOTOXICITY

28

Singh H, Singh JR, Dhillon VS, Bali D, Paul H. IN VITRO AND IN

VIVO GENOTOXICITY EVALUATION OF HORMONAL DRUGS. II. DEXAMETHASONE
Mutat Res 1994;308(1):89-97.

Genotoxicity evaluation of a widely used glucocorticoid medicine, dexamethasone, was undertaken using in vitro and in vivo assays. Analyses of chromosomal aberrations, sister-chromatid exchanges (SCEs) in human lymphocytes and micronuclei and SCEs in mouse bone marrow showed the drug to be capable of attacking the genetic material. However, the Ames/Salmonella assay, both with and without S9 mix, did not show any increase in His+ revertants.

29

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

Sodium arsenite (NaAsO2) and cadmium sulfate (CdSO4) were tested for their ability to induce genotoxic effects in the single cell gel (SCG) assay and the sister chromatid exchange (SCE) test in human blood cultures in vitro. Both metals induced DNA damage in white blood cells that was expressed and detected as DNA migration in the SCG assay. Dose dependent effects were seen for cadmium in concns. from 5 .times. 10-4-5 .times. 10-3 M and for arsenic in concns. from 2 .times. 10-4-1.5 .times. 10-3 M. The distribution of DNA migration among cells, a function of dose, revealed that the majority of exposed cells expressed more DNA damage than cells from control cultures and that with increasing length of DNA migration the variability in migration among cells increased as well. Treatment of cells for 2 h or 24 h beginning 48 h after the start of the blood cultures did not increase the SCE frequency in the case of cadmium but caused a small but significant SCE induction with arsenic at the highest concn. The metal concns. which could be investigated in the SCE test were much lower due to a strong toxic effect. Metal concns. which were toxic in the SCE test were without visible effect in the SCG assay. Thus the two endpoints for the detn. of genotoxic effects in vitro differed markedly with respect to the detection of genotoxicity induced by metals. These differences and the biol. significance of the findings are discussed.

30
Paolini M, Biagi GL, Bauer C, Cantelli-Forti G. ON THE PROCEDURES FOR ISOLATION OF S9 FRACTIONS FROM INDUCED RODENTS IN IN VITRO GENOTOXICITY ASSAYS. Teratogen Carcinogen Mutagen 1994; 14(2):101-103.

No abstract.

31
Gallaway SM. CHROMOSOME ABERRATIONS INDUCED IN VITRO: MECHANISMS, DELAYED EXPRESSION, AND INTRIGUING QUESTIONS. Environ Molec Mutagen 1994;23(Suppl 24):44-53.

Chromosome aberrations including breakage and rearrangement and numerical changes, are important in carcinogenesis, heritable mutations, embryonic loss, and developmental abnormalities. We can detect DNA reactive agents in in-vitro chromosome aberration assays, but aberrations are also induced by chemicals that do not directly interact with DNA. This article discusses briefly some important aspects of using aberrations in genetic toxicology testing but concentrates on highlights of recent research on aberrations, in particular two areas: (1) persistence through multiple cell cycles of changes that lead to chromosome aberrations, and (2) the relations among DNA synthesis

inhibition, DNA damage, cell cycle regulation, and genomic instability, expressed as chromosome breakage, gene amplification, and aneuploidy. An understanding of these mechanisms not only may lead to insights into carcinogenesis but ultimately may help us to interpret results of chromosome aberration tests and to develop a rational assessment of the degree of human risk implied by a positive aberration test.

32
Anon. GENOTOXICITY STUDIES OF MILD GASIFICATION PRODUCT, MRE(NUMBER SIGN)1, IN MAMMALIAN CELLS. (Quarterly technical progress report, July 1, 1993--September 30, 1993). Department of Energy, Washington, DC. Govt Repts Announcs & Index (GRA&I), Issue 14, 1994.

The major hypothesis of carcinogenesis is that malignancy is due to an alteration (mutation) of the genetic material in a somatic cell. Reactive electrophilic metabolites are generated from many chemicals by the action of endogenous mixed function oxidases. These reactive metabolites may bind to cellular macromolecules such as DNA, and can, therefore, initiate a mutagenic or carcinogenic event. Prokaryotes and non-mammalian eukaryotes are used in mutation assays, while cultured mammalian cells are generally used for mutagenic as well as clastogenic tests examining alterations and damage to the DNA and/or chromosomes of somatic cells. One of the first mammalian cell lines used in genotoxicity studies is V79, which was derived from Chinese hamster lung cans. According to the test plan on toxicity studies of mild gasification products, mammalian cell in vitro assays are to be performed on selected samples displaying mutagenic activity in the Ames assay. The results of the Ames testing of the mild gasification sample MRE(number sign)1 indicate weak, but significant mutagenic activity. Hence, assays for the induction of gene mutation, sister chromated exchange and micronucleus formation in V79 cells have been carried out for the sample. This paper reports the results of these assays.

33
Anon. TOXICITY STUDIES OF MILD GASIFICATION PRODUCTS: QUARTERLY TECHNICAL PROGRESS REPORT, APRIL--JUNE 1993. Department of Energy, Washington, DC. Govt Rept Announc & Index (GRA&I), Issue 13, 1994.

The major hypothesis of carcinogenesis is that malignancy is due to an alteration (mutation) of the genetic material in a somatic cell. Reactive electrophilic metabolites are generated from many chemicals by the action of endogenous mixed function oxidases. These reactive metabolites may bind to cellular macromolecules, such as DNA, and can, therefore, initiate a

mutagenic or carcinogenic event. Prokaryotes and non-mammalian eukaryotes are used in mutation assays, while cultured mammalian cells are generally used for mutagenic as well as clastogenic tests examining alterations and damage to the DNA and/or chromosomes of somatic cells. One of the first mammalian cell lines used in genotoxicity studies is V79, which was derived from Chinese hamster lung cells. According to the test plan on toxicity studies of mild gasification products, mammalian cell in vitro assays are to be performed on selected samples displaying mutagenic activity in the Ames assay. The results of the Ames testing of the mild gasification sample IST (number sign)10 indicate significant mutagenic activity. Hence, assays for the induction of gene mutation, sister chromatid exchange and micronucleus formation in V79 cells have been carried out for the sample. This paper reports the results of these assays.

34
Piatti E, Marabini L, Chiesara E. INCREASE OF MICRONUCLEUS FREQUENCY IN CULTURED RAT HEPATOCYTES TREATED IN VITRO WITH BENOMYL AND PIRIMIPHOS-METHYL SEPARATELY AND IN MIXTURE. Mutat Res 1994;324(1-2):59-64.

The pesticides benomyl, a benzimidazole fungicide, and pirimiphos-Me, an organophosphorus insecticide, were tested sep. and in combination at a ratio of 6 : 1, a mixt. frequently found in foodstuffs by residual anal., to det. their possible genotoxic action. The effect was measured by the micronucleus test carried out on cultured rat hepatocytes stimulated to proliferate by epidermal growth factor (EGF). Adult rat hepatocytes were exposed in vitro for 48 h to the substances at increasing non-cytotoxic doses, chosen on the basis of cytotoxicity tests such as LDH and Neutral red assays. Benomyl induced a significant dose-related increase in micronucleus frequency; in contrast, pirimiphos-Me was not genotoxic at any dose tested. When the hepatocytes were exposed to the two pesticides together at increasing doses, an enhancement in micronucleus frequency similar to that of benomyl alone was found, indicating that at this ratio and non-cytotoxic doses (up to 25 mug/mL benomyl+4.2 mug/mL pirimiphos-methyl) no interaction occurs.

35
Kopponen P, Torronen R, Maki-Paakkanen J, von Wright A, Karenlampi S. COMPARISON OF CYP1A1 INDUCTION AND GENOTOXICITY IN VITRO AS INDICATORS OF POTENTIALLY HARMFUL EFFECTS OF ENVIRONMENTAL SAMPLES. Arch Toxicol 1994;68(3):167-73.

Cytochrome P450IA1 (CYP1A1) induction of Hepa-1 mouse and H4IIE rat hepatoma cell lines was compared using selected environmental samples. The results were in agreement for both cell lines: no

induction was observed for the fly ash extract from peat combustion, an intermediate induction was found for the fly ash extract from biosludge combustion, and a strong induction was detected for natural peat extract. However, Hepa-1 responded to the samples more sensitively than did H4IIE: the half maximal induction (ED50) values for Hepa-1 were smaller than those for H4IIE. In a bacterial DNA repair assay without metabolic activation and in a mammalian sister chromatid exchange test in the presence of metabolic activation the samples were virtually non-genotoxic. Thus the CYP1A1-inducing potency and genotoxicity of the samples were not correlated. In light of these results, the CYP1A1 induction test might be a useful addition to conventional genotoxicity tests, which may fail to detect potentially harmful compounds/mixtures.

HEMATOTOXICITY

36
Yamaguchi F, Furuhama K, Miyamoto M, Sagara-Ishijima N, Takayama S. APPLICATION OF HEMATOPOIETIC PROGENITOR ASSAYS FOR THE ESTIMATION OF HEMATOXICITY IN RATS. J Pharmacol Toxicol Methods 1994;31(2):71-7.

In a study to employ progenitor-derived clonogenic assays of granulocyte-macrophage colony-forming units (CFU-GM) and erythroid colony-forming units (CFU-E) for the assessment of chemically induced hematotoxicity in rats, we sought the appropriate culture medium for each assay. Then, the effect of cyclophosphamide or phenylhydrazine on bone marrow cells was examined in vivo and in vitro. Oral treatment of rats with 25 mg/kg of cyclophosphamide significantly decreased the number of CFU-GM, which was an earlier and more sensitive index than were other hematological parameters tested. Direct exposure of the culture to cyclophosphamide had little effect on CFU-GM production, but addition of sera from rats pretreated with cyclophosphamide strongly depressed their formation, which suggested that the active metabolites of cyclophosphamide produced in the body may play an important role in toxicity. Subcutaneous injection of rats with 40 mg/kg of phenylhydrazine produced a marked increase in CFU-E 6-24 hr after injection. Direct exposure of the culture to phenylhydrazine had severe cytotoxic effects, but addition of sera from rats receiving phenylhydrazine resulted in increased numbers of CFU-E, probably indicative of increases in endogenous erythropoietin in donor rats. These results demonstrate that the proper use of progenitor assays in the in vivo and in vitro studies may be a valuable tool for approaching the mechanism underlying hematotoxicity.

37

Parent-Massin D, Thouvenot D, Rio B, Riche C. LINDANE HEMATOTOXICITY CONFIRMED BY IN VITRO TESTS ON HUMAN AND RAT PROGENITORS. Hum Exp Toxicol 1994;13(2):103-6.

Culture of hematopoietic progenitors, Colony Forming Unit-Granulocyte and Macrophage (CFU-GM), have been performed in the presence of lindane with increasing concns. Results showed that lindane was cytotoxic for human progenitors. They were one thousand times more sensitive to the lindane than rat CFU-GM. This cytotoxicity was obsd. with lindane concns. similar to those measured in human blood in cases of acute intoxication and in fat tissues of exposed populations.

HEPATOTOXICITY

38

Fernyhough L, Kell SW, Hammond AH, Thomas NW, Fry JR. COMPARISON OF IN VIVO AND IN VITRO RAT HEPATIC TOXICITY OF COUMARIN AND METHYL ANALOGUES, AND APPLICATION OF QUANTITATIVE MORPHOMETRY TO TOXICITY IN VIVO. Toxicol 1994;88(1-3):113-125.

The rat hepatic toxicity of coumarin and methyl analogues (3-, 4-methyl coumarin and 3,4-dimethylcoumarin) has been determined in vivo and in vitro (freshly-isolated cells). Coumarin at a dose of histological evidence of centrilobular necrosis, while the methyl analogues at an equivalent dose were much less toxic. By use of a systematic random sampling protocol and quantitative morphometry it was determined that there was a lobar variation in the extent of hepatic damage but that this exhibited random inter-animal variation. The order of cytotoxicity in vitro was identical to that observed in vivo. In hepatocytes depleted of glutathione the toxicity of all four compounds was increased. This was particularly marked for the 3-methyl analogues, such that the order of toxicity was different to that observed in vivo and in hepatocytes not depleted of glutathione.

IMMUNOTOXICITY

39

Fuchs BA, Sanders VM. THE ROLE OF BRAIN-IMMUNE INTERACTIONS IN IMMUNOTOXICOLOGY. Crit Rev Toxicol 1994;24(2):151-76. (201 REFS)

Certain xenobiotics (or the metabolites) can damage immunocompetence by directly interacting with one or more of the cells of the immune system and adversely affecting its function. It has also been proposed that xenobiotics may indirectly affect immune function by affecting other organ systems that will in turn affect immunocompetence. This review surveys evidence that supports the existence of a functional link between the brain and the immune system. In addition, we review data that support the concept that a xenobiotic-induced dysfunction in the

neuroendocrine system may be associated with an immune dysfunction as well. Such chemicals do not necessarily interact directly with immunocompetent cells but would instead act to disrupt regulatory brain-immune interactions. This class of indirectly acting immunotoxic xenobiotics would not be detected in the typical in vitro screening assays.

MECHANISMS/TOXICITY
40
De Angelis I, Hoogenboom L AP, Huveneers-Oorspoong M BM, Zucco F, Stammati A. ESTABLISHED CELL LINES FOR SAFETY ASSESSMENT OF FOOD CONTAMINANTS: DIFFERING FURAZOLIDONE TOXICITY TO V 79, HEp-2 AND Caco-2 CELLS. Food and Chem Toxicol 1994; 32(5):481-488.

In vitro models, preferentially derived from human tissues, may be valuable tools to study the biotransformation and toxicity of compounds that may be present as residues in food products. Such residues may represent a risk to human health, and therefore call for increased testing. Three established cell lines were used to study the toxic effect of furazolidone (FZ), a widely used veterinary drug: HEp-2 cells, derived from a human larynx carcinoma, previously used in toxicity screening of several compounds; Caco-2 cells, derived from a human colon enocarcinoma, able to differentiate partially in culture, and V 79, a fibroblast cell line derived from Chinese hamster lung, widely used to assess direct toxicants. Various toxicity parameters were used, primarily dealing with cell death and cell proliferation. In all cell lines FZ at a concentration of 5 mug/ml caused a marked decrease in cell viability and especially in cell proliferation. Inhibition of DNA synthesis has also been observed, even if at higher concentrations. However, only in V 79 cells was the decrease in cell number accompanied by a marked increase in lactate dehydrogenase leakage due to membrane damage. Moreover, the surviving V 79 cells, after removal of FZ, fully recovered from the effect of the drug, as shown by their full capacity to attach to dishes and to form colonies. Surviving cells of the other two cell lines showed much poorer colony-forming ability. Exposure of Caco-2 cells and, to a lesser extent, HEp-2 cells, caused a marked increase in oxygen consumption, that possibly was due to redox cycling of the initially formed radical nitro anion. Biotransformation of the drug by all three cell lines was accompanied by the formation of protein-bound metabolites, HEp-2 being the most active cells. The toxic effects recorded show that cell lines provide a sensitive system in toxicity assessment. Moreover, it may be suggested that a battery of cell lines, including some of human origin, as well as a battery of endpoints, may be of help in addressing further specific mechanistic investigations.

METABOLISM/TOXICITY

41

Maurel P. DRUG METABOLISM IN PRIMARY CULTURES OF ADULT HUMAN HEPATOCYTES. Meeting of the Tissue Culture Association on Regulation of Cell and Tissue Differentiation, Research Triangle Park, North Carolina, USA, June 4-7, 1994. In Vitro Cellular & Developmental Biology Animal 1994;30A(3 Part 2):38.

No abstract.

MUTAGENESIS

42

Madle S, Dean SW, Andrae U, Brambilla G, Burlinson B, Doolittle DJ, Furihata C, Hertner T, McQueen CA, et al. RECOMMENDATIONS FOR THE PERFORMANCE OF UDS TESTS IN VITRO AND IN VIVO. Mutat Res 1994;312(3):263-85.

The Working Group (WG) dealt with the harmonization of routine methodologies of tests for unscheduled DNA synthesis (UDS) both in vitro and in vivo. In contrast to the existing guidelines from OECD, EPA and EC on in vitro UDS tests (there is no Japanese UDS guideline), the Working Group recommends that in general in vitro UDS tests should be performed with primary hepatocytes. For routine applications any other cell types would need special justification. Hepatocytes from male rats are preferable, unless there are contra-indications on the basis of e.g. toxicokinetic data. According to the OECD, EPA and EC guidelines, UDS may be analyzed by means of autoradiog. (AR) or liq. scintillation counting (LSC). The WG recommends use of AR. LSC is less suitable due to the problem of differentiation between UDS activity and replicative DNA synthesis, and the disadvantage that cells cannot be analyzed individually. Since a specific cell type was recommended by the WG, methodology aspects could be described in more detail than in the present guidelines. For in vitro tests, it was agreed that the initial viability of freshly isolated hepatocytes should be at least 70%. With regard to the need for confirmatory expts. in the event of a clear-cut negative result, the majority view was that confirmation by a second (normally not identical) expt. is still needed; this is in line with the present OECD and EC guidelines. Evaluation of results from UDS tests should be based primarily on net nuclear grain (NNG) values, although it is recognized that nuclear and cytoplasmic grains result from different biol. processes. Since grain counts are influenced by a number of methodol. parameters, no global threshold NNG value can be recommended for discrimination of pos. and negative UDS results. For in vitro assays, the criteria for positive findings go beyond those of the present guidelines and two alternative approaches are given which are based on (1) dose-dependent increases in NNG values and (2) reproducibility,

dose-effect relationship and cytotoxicity. At present there is no official guideline on the performance of in vivo UDS tests. Some fundamental recommendations given for in vitro methodololgy also apply to the in vivo assay. For routine testing with the in vivo UDS test, again the general use of hepatocytes from male rats is recommended. However, concerning the requirement to use one or two sexes, consistency with other in vivo genotoxicity assays (e.g. the micronucleus assay) would be preferable. As for the in vitro methodology, AR is preferred rather than LSC. For in vivo UDS tests, a min. viability of 50% is considered to be sufficient. Sampling of cells 12-16 h after treatment and, if this is neg., 2-4 h is recommended. At least three animals per treatment group should be used. Evaluation of results should again be done on the basis of NNG values. (Abstract truncated at 400 words)

43
Gatehouse D, Haworth S, Cebula T, Gocke E, Kier L, Matsushima T, Melcion C, Nohmi T, Ohta T, et al. RECOMMENDATIONS FOR THE PERFORMANCE OF BACTERIAL MUTATION ASSAYS. Mutat Res 1994; 312(3):217-33.

At the International Workshop on the Standardization of Genotoxicity Test Procedures, in Melbourne (27-28 Feb. 1993), the current international guidelines for the correct conduct of bacterial mutation assays were considered, and the major differences between them were examd. An attempt was made to construct a scientifically based, internationally harmonized protocol. The main points of agreement were as follows. The consensus opinion was that there are currently insufficient data to justify a preference for either the preincubation or plate-incorporation methodologies as the initial test. Whichever method is used there was consensus agreement that the bacterial test battery should consist of S. typhimurium TA1537, TA1535, TA98 and TA100. There was also consensus that the 3 strains TA97a, TA97 and TA1537 could be used interchangeably. Although it was not possible to achieve a consensus, the majority of the working group members agreed that strains for the detection of mutagens acting specifically on AT base pairs should be routinely included within the test battery. These strains may be S. typhimurium TA102 or E. coli WP2 strains (WP2 pKM101 and WP2 uvrA or WP2 uvrA pkM101). With regard to study design it was universally agreed that 5 doses of test compd. should be used in each expt., and a majority agreement was obtained for 3 plates per dose. The use of 2 plates per dose is acceptable ONLY if the expt. is repeated. It is recommended that the neg. controls may consist of solvent control alone provided that historical data are available to demonstrate lack of effect of the solvent in question. Pos. control compds. should be included in all expts., although the nature of these control compounds need not be

specified in the guidelines. There was consensus agreement that for non-toxic freely sol. test agents, an upper limit of 5 mg/plate should be tested (5 muL per plate for liqs.). For insol. or toxic compounds, the recommendations were the same as those for other in vitro tests (see appropriate paper). A consensus agreement was reached on the need to carry out further tests if equivocal results are obtained in the initial test, although it was generally agreed that the design of the repeat study should be left flexible. As there are little or no data to support the use of an exact repeat assay, a majority of the group recommended that neg. results in the first test should be further investigated by either conducting a modified repeat (e.g. S9 titrn.) or by conducting the alternative methodol. If a preincubation assay is carried out, an incubation time within the range 20-60 min is recommended, usually at a temp. of 37.degree.C. Lastly, a consensus agreement on the acceptable criteria for a pos. or neg. result could not be reached. There was majority agreement that a reproducible dose-response was necessary for a chem. to be classified as pos. (Abstract truncated at 400 words,)

44
Shimizu H, Suzuki Y, Okonogi H. COMPARATIVE STUDY OF THE IN VITRO MICRONUCLEUS TEST USING A CHINESE HAMSTER CELL LINE AS A SHORT TERM TEST. Hen'igensei Shiken 1994;3(1):41-8.

The results of the in vitro micronucleus (MN) test using a Chinese hamster lung cell line (CHL) were evaluated on 76 clastogenic, mutagenic or carcinogenic compounds. Thirty one out of 76 compds. were the results of a collaborative study sponsored by the Ministry of Labor and the rest was the authors' data. The results of the MN test on 76 compds. were compared with the results of structural aberration and polyploidy of conventional chromosomal aberration test, qual. All chems. reported induced micronuclei. The authors conclude that the in vitro MN test is useful for the detection of clastogenic chems. as a screening test.

NEUROTOXICITY
45
Flowers AE, Capra MF, Cameron J. THE EFFECTS OF CIGUATOXIN ON THE NEVERS OF THE TELEOST FISH, SILLAGO CILIATA. Natural Toxins 1992;1(2):126-135.

The absolute refractory period, relative refractory period, and the duration and magnitude of the supernormal period were measured after incubation of fish nerves with ciguatoxin and other channel modifying compounds, tetrodotoxin, veratridine,

verapamil, and lignocaine. In vitro electrophysiological studies were carried out on the lateral line nerve of the whiting, Sillago ciliata Cuvier. Electrophysiological changes in fish nerves after exposure to ciguatoxin (0.3 MU.ml-1) and veratridine (occur in mammalian nerves and include an increase in the absolute refractory period, the relative refractory period, and the magnitude and duration of supernormailty. The effects of ciguatoxin (0.3 MU.ml-1) in fish nerves were antagonized by tetradotoxin (50-5 g/ml-1). The nerves of Sillago ciliata used in this study responded to ciguatoxin and its antagonists in a similar manner to mammalian nerves, suggesting that these teleost nerves have no specific electrophysiological mechanism to cope with this toxin.

46
Tuttle JB, Mackey T, Steers WD. NGF, bFGF and CNTF INCREASE SURVIVAL OF MAJOR PELVIC GANGLION NEURONS CULTURED FROM THE ADULT RAT. Neurosci Lett 1994;173(1-2):94-8.

The responsiveness of cultured major pelvic ganglion (MPG) neurons, isolated from adult rats, to nerve growth factor (NGF), basic fibroblastic growth factor (bFGF) and ciliary neuronotrophic factor (CNTF) was tested using in vitro survival assay. MPG neurons respond to NGF with increased survival (+35%, mean .+-. S.E.), a response completely blocked by antibodies specific to NGF. bFGF (+85%) and CNTF (+10.5%) also augment survival of MPG neurons in vitro. The effect of bFGF was partially blocked by bFGF antibody. Anti-NGF antibody reduced neuronal survival by 25% in conditioned medium from cultures of bladder smooth muscle, suggesting bladder produces NGF. Combining antibodies against NGF and bFGF reduced survival by 19% in medium supplemented with bladder exts., suggesting the exts. contain neurotrophic activity in addn. to NGF. These results support the hypothesis that neurons regulating bladder function respond to NGF and other growth factors. Therefore, previously documented changes in bladder neurotrophic factors following hypertrophy, inflammation and injury may elicit growth or change in the autonomic nervous system.

47
Flint OP, Weiss A, Durham SK. ANTI-AIDS NUCLEOSIDE ANALOGS (ddI, d4T, ddC, and AZT): COMPARATIVE IN VITRO NEUROTOXICITY STUDY USING THE MICROMASS CULTURE TECHNIQUE. In Vitro Toxicol 1993; 6(4):221-41.

Sensory peripheral neuropathy has been observed in phase I studies in human immunodeficiency virus (HIV)-infected patients treated with the nucleoside analogs 2',3'-dideoxycytidine (ddC), 2',3'-didehydro-3'deoxythymidine (d4T), and 2',3'-dideoxyinosine

(ddI). The neuropathic potency in humans is ddC > d4T > ddI. A sensory neuropathy has not been observed in ddI- or d4T-treated primates, dogs, rabbits, rats, or mice, but a sensorimotor neuropathy was reported in ddC-treated rabbits. The objectives of the present study were to characterize the concentration-toxicity profiles of the synthetic nucleoside analogs, using cultures of differentiating or differentiated neurons; and to compare the synthetic nucleoside analogs with the naturally occurring deoxynucleosides (deoxycytidine [dC], thymidine [dT], and deoxyinosine [dI]) and with 3'-azido-2',3'-deoxythymidine (zidovudine, AZT). Thirteen-day (post coitum) rat embryo midbrain cells were cultured as micromass "cell islands.". During the first 5 days of culture, cells differentiate into distinct foci of neurons surrounded by areas of undifferentiated cells. Length of drug exposure and the amt. of time after initial culture before drug exposure were the main variables. Endpoints measured following exposure to test agent were viability of differentiated and undifferentiated cells (neutral red assay), neuron-specific differentiation (image anal. of foci of differentiated neurons), energy status (cellular ATP), and ultrastructural morphol. (electron microscopy). A similar order of toxicity in vitro, as compared to the neuropathic potency in vivo, was obsd. in cells exposed to test agent for 24 h followed by a further 4 days in test-agent-free medium. The naturally occurring deoxynucleosides (dC, dT, and dI) were all markedly less toxic than their dideoxy counterparts (ddC, d4T, and ddI). When compared to other in vitro systems, micromass cultures of rat embryo neurons rank the synthetic nucleosides in order of their clin. neuropathic potency. This culture system may be useful in predicting the toxicity of novel synthetic analogs, or in further studies of the mechanism of the neuropathy.

48
Castillo BJ, del Cerro M, Breakefield XO, Frim DM, Barnstable CJ, Dean DO, Bohn MC. RETINAL GANGLION CELL SURVIVAL IS PROMOTED BY GENETICALLY MODIFIED ASTROCYTES DESIGNED TO SECRETE BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF). Brain Res 1994;647(1):30-6.

Genetically engineered cells carrying genes for neurotrophic factors have potential application for treatment of neurodegenerative diseases and injuries to the nervous system. Brain-derived neurotrophic factor (BDNF) promotes the survival of specific neurons, including retinal ganglion cells (RGC). To det. whether genetically engineered astrocytes might be use for delivering bioactive BDNF, the authors infected primary type 1 rat astrocytes with a retrovirus harboring a human prepro-BDNF cDNA and assayed the medium conditioned by these astrocytes for effects on survival of rat RGCs in vitro. High levels of BDNF mRNA were expressed by infected astrocytes, but not by control astrocytes as determined by RNase protection assay using a BDNF

specific probe. To test for secretion of bioactive BDNF from the transgenic astrocytes, embryonic day 17 rat retinas were dissocd. and grown in medium conditioned (CM) for 24 h by astrocytes infected with a replication deficient retrovirus carrying BDNF, NGF, or alk. phosphatase (AP) cDNA. After 3 days, the number of Thy-1 immunoreactive RGCs was counted. BDNF astrocyte CM significantly enhanced RGC survival by 15-fold compared to AP control. NGF astrocyte CM had no significant effect. The rate of BDNF secretion was estd. at 83-166 pg/105 cells/h. This study demonstrate that astrocytes can be genetically engineered to synthesize and secrete bioactive BDNF. These techniques may be applicable to rescuing neurons from degenerative processes and also for enhancing their survival following transplantation.

OCULAR TOXICITY

49
Bagley D, Booman KA, Bruner LH, Casterton PL, Demetrulias J, Heinze JE, Innis JD, McCormick W C III, Neun DJ, Et al. THE SDA ALTERNATIVES PROGRAM PHASE III: COMPARISON OF IN VITRO DATA WITH ANIMAL EYE IRRITATION DATA ON SOLVENTS, SURFACTANTS, OXIDIZING AGENTS, AND PROTOTYPE CLEANING PRODUCTS. J Toxicol Cutaneous Ocul Toxicol 1994;13(2):127-155.

Nine in vitro candidate tests for estimating eye irritation potential were evaluated as potential replacements for the Draize test. The tests examined were a cell protein assay, the chorioallantoic membrane vascularization assay, a cell protein assay, a fibroblast cytotoxicity assay, the Living Dermal Model and Living Skin Equivalent, two neutral red assays, an SIRC cytotoxicity assay, and a Tetrahymena thermophila motility assay. The results from these in vitro tests were compared to results from a modified Draize test with 22 test materials. The test materials were selected to represent various classes of cleaning products and ingredients. Ingredients were tested at concentrations representative of concentrations typically found in cleaning products. The correlation coefficients with all test materials considered ranged from 0.58 to 0.91. When only nonalkaline materials are considered, the correlation coefficients of all 10 tests were not significantly different from one another, r of the test substances were the corneal epithelial plasminogen activator assay, the chorioallantoic membrane vascular assay, and the Tetrahymena mortality assay. Further, six of the 10 tests were able to identify the five nonirritants in the study, although the relative irritation potentials of the irritants were not accurately predicted by any of the tests. Results from a low-volume eye irritation test (LVET) were also compared to results from a modified Draize test with the same 22 test materials. The LVET had a high correlation with the modified Draize test and will be useful for future comparison with other alternative eye irritation tests. Based on

these data, a number of alternative tests developed to replace the Draize eye irritation test included in this phase of research are useful for screening the eye irritation potential of nonalkaline cleaning products, although some tests are better for identifying the eye irritation potential of test materials with alkaline or oxidation potential. Although the ability of the eye to recover from damage was not measured by any test, the tests show promise for the use of determining eye irritation potential.

50
Hayashi T, Itagaki H, Fukuda T, Tamura U, Kato S. MULTIVARIATE FACTORIAL ANALYSIS OF DATA OBTAINED IN SEVEN IN VITRO TEST SYSTEMS FOR PREDICTING EYE IRRITANCY. Toxicol In Vitro 1994;8(2):215-220.

Seven in vitro test systems used to predict eye irritancy (EYTEX, SIRC cytotoxicity, HeLa cytotoxicity, chorioallantoic membrane (CAM), liposome, red blood cell and haemoglobin denaturation test system) were applied to 12 surfactants, and the results were subjected to multivariate analysis to evaluate the relative contributions of five factors. These factors were: (1) cellular plasma membrane destruction factor, (2) haemoglobin type protein denaturation factor, (3) EYTEX-type protein denaturation factor, (4) cytotoxicity factor and (5) an unknown (unidentified) factor. The results clarified the basis on which the findings of each test system were related to the Draize results. According to the analysis, the Draize eye irritation test could be explained by the contribution of the protein denaturation factor and cellular plasma membrane destruction factor. This provides support for a previous hypothesis that the major mechanisms of eye irritation are cellular plasma membrane destruction and protein denaturation. The CAM test value showed a higher correlation coefficient (0.906) with the Draize score than did the results of any of the other test systems; this was due to the fact that these two tests showed similar patterns of dependence on the five factors as indicated by the factorial analysis. The haemoglobin denaturation test system had the next highest correlation coefficient at about 0.75. Furthermore, by using further tests to make up the deficiency of the other necessary factors, a desirable battery system could be predicted; for example, the combination of the haemoglobin-denaturation and rat red blood cell tests would provide a result similar to that of the Draize test. This study should contribute to the development of a rational basis for prediction of eye irritancy of chemicals using in vitro test systems.

51
O'Brien KA F, Basketter DA, Jones P, Dixit M. AN IN VITRO STUDY OF THE EYE IRRITATION POTENTIAL OF NEW SHAMPOO FORMULATIONS.

Toxicol in Vitro 1994;8(2):257-61.

Several in vitro methods were used to investigate the toxicity of potential eye irritants with a view to replacing in vivo eye irritation testing. Initially, a group of surfactant and surfactant-based products, for which inhouse in vivo Draize eye irritation data were already available, were studied in a range of in vitro assays. This approach obviated the need to generate in vivo data specifically to evaluate the current in vitro program. Chinese hamster V79 cells, mouse peritoneal macrophages, mouse Balb/c3T3 cells and mouse L929 fibroblasts were used together with several different endpoints of toxicity, including dye retention techniques, reductive capacity and morphol. assessment. In addn., the samples were tested in a bacterial bioluminescence system, Microtox. The data from previous studies indicated that the best agreement with irritancy data was obtained using a test battery consisting of fluorescein diacetate incorporation by V79 cells, neutral red uptake by 3T3 cells the L929 agarose overlay assay and the Microtox assay. This report presents an example of the use of this in vitro evaluation process for surfactant-based personal products. In each case, the in vitro toxicity of the new formulation was compared with that of a range of related products for which historical eye irritation data, generated using either

the Draize test in vivo or the enucleated eye assay in vitro, were available. The data for each in vitro assay were evaluated by comparing the toxicity rank order with the available historical data. In general, the in vitro ranking was in good agreement with the historical data, although the L929 assay proved to be of limited value. This approach allowed the completion of the safety evaluation process for the novel formulations.

52
Donnelly T, Decker D, Stemp M, Rheins L, Logemann P. ASSESSMENT OF THE OCULAR IRRITANCY POTENTIAL OF HAIR CONDITIONERS IN A THREE-DIMENSIONAL IN VITRO HUMAN SKIN MODEL. J Toxicol, Cutaneous Ocul Toxicol 1994;13(2):117-25.

A 3-dimensional human tissue model was developed in the authors' lab. and used as a substrate for assessing ocular cytotoxicity and irritancy potential. The full-thickness model is a coculture of dermal fibroblasts and epithelial cells (keratinocytes). Metabolically and mitotically active fibroblasts were seeded onto medical-grade nylon mesh, where they attach and secrete collagen and extracellular matrix proteins. Then keratinocytes were seeded onto the top of this submerged stromal tissue, and the tissue grows into a multilayered epithelium. Histol. examn. of this cellular coculture system reveals a cellular organization similar

to the rabbit/human cornea. The resulting substrate has been used effectively to study the effects of a variety of test compounds (surfactants, powders, creams) at dild. or full-strength concns. in a time-course assay. The protocol mimics the way in which in vivo animal testing (Draize eye testing) is performed. The treated tissue is assayed for cytotoxicity over a period of time. The high correlation (r = 0.87) of the authors' in vitro data with existing animal eye data shows the potential usefulness of these tissue substrates as an in vitro alternative for evaluating the toxicity of hair care formulations.

53
Gautheron P, Duprat P, Hollander CF. INVESTIGATIONS OF THE MDCK PERMEABILITY ASSAY AS AN IN VITRO TEST OF OCULAR IRRITANCY. In Vitro Toxicol 1994;7(1):33-43.

A recent in vitro assay investigated the increased permeability of injured ocular tissues by monitoring the passage of fluorescein through a confluent monolayer of Madin-Darby Canine Kidney (MDCK) cells. A disruption of the epithelial barrier was obsd. in parallel with the irritant potential of the test substances. The objective of the present study was to det. the usefulness of this test as a method for screening process intermediates, with a set of 42 ref. chems. Surfactants were the most active substances with a ranking similar to in vivo ocular irritancy. Among the other chems. tested, the most potent in vitro were classified as severely irritating in vivo, but the reverse was not always true. In addn., moderate and mild irritants appeared to be poorly differentiated in vitro. For surfactant and misc. chems., fluorescein leakage correlated better with the in vivo conjunctival score ($r > 0.9$) than with the in vivo corneal score ($r < 0.7$). In contrast, alcs. were better correlated with the in vivo corneal score ($r = 0.87$, as compared to $r = 0.53$ for the conjunctival score). A strong correlation ($r = 0.98$) was also obsd. between fluorescein permeability values and partition coeffs. for a group of 13 alcs. and 3 ketones. This assay therefore appears to be very useful for predicting the potential irritancy of surfactants, but seems less predictive for the other categories of chems., at least with the methodol. employed. Finally, hydrophobic properties of chems. may play a direct role in this test system as in some other cell culture-based assays.

54
Takahashi H, Palcic B, Damji KF, Rootman J, Drance SM. ASSESSMENT OF ANTI-PROLIFERATIVE AND ANTI-INFLAMMATORY DRUGS FOR TREATMENT OF OCULAR FIBROBLAST PROLIFERATION USING DYNAMIC MICROSCOPE IMAGE PROCESSING SCANNER. Nippon Ganka Gakkai Zasshi 1993;97(1):3-10.

Controlling ocular fibroblast proliferation may significantly improve the effectiveness of glaucoma filtration surgery. Drugs for the inhibition of fibroblast growth are currently in clin. use. However, systemic studies of drugs with different mechanisms of action have not been performed on human ocular fibroblasts. The authors have developed a method to evaluate the effect of chemotherapeutic agents on fibroblast proliferation, motility, and dynamic morphol. This involves the use of an automated microscope system designed for quant. measurement of movement and morphol. of live cells in tissue culture. This technique was tested on an established tissue culture fibroblast (3T3 cells) and then applied to secondary culture of human scleral and subconjunctival fibroblasts. 5-Fluorouracil and colchicine were tested for their effect on fibroblast behavior. The authors' data indicated that colchicine was more effective than 5-fluorouracil in inhibiting fibroblast proliferation and movement, and in changing morphol. Using this system, fundamental biol. effects of various pharmacol. manipulations could be studied in vitro prior to in vivo applications.

55
Vanparys P, Deknudt G, Sysmans M, Teuns G, Coussement W, Van Cauteren H. EVALUATION OF THE BOVINE CORNEAL OPACITY-PERMEABILITY ASSAY AS AN IN VITRO ALTERNATIVE TO THE DRAIZE EYE IRRITATION TEST. Toxicol in Vitro 1993;7(4):471-6.

The bovine corneal opacity-permeability assay (BCO-P) was evaluated as an in vitro alternative test model for the Draize eye irritancy test. Fifty pharmaceutical and com. available compounds were tested in the BCO-P assay. The compounds were selected on the basis of their in vivo irritancy potential as determined in previous Draize tests. Liquids as well as solids were tested. Corneal opacity and permeability were measured to determine ocular irritation potential. When two irritancy classifications (non-irritant and irritant) were considered, 96% of the tested chemicals were classified correctly. A 72% concordance was obtained when four irritancy classifications (non-irritant, mild, moderate and severe irritant) were considered. Furthermore, all compounds that were severe eye irritants in vivo were equally scored in vitro. The results of this study show that the BCO-P assay is a competent in vitro test system for the prediction of ocular irritation of chemicals. This test model can be used as a first screen to avoid in vivo testing of severe ocular irritants.

56
Grant RL, Acosta D. COMPARATIVE TOXICITY OF TETRACAINE, PROPARACAINE AND COCAINE EVALUATED WITH PRIMARY CULTURES OF

RABBIT CORNEAL EPITHELIAL CELLS. Exp Eye Res 1994;58(4):469-78.

Cocaine was first used as a topical anesthetic for the eye by Dr. Carl Koller in 1884. It became evident that this agent produced erosion of the corneal epithelium in high doses or with repeated use. Synthetic local anesthetics such as tetracaine and proparacaine were developed which were more potent and less toxic than cocaine, but still produced corneal epithelium defects if used chronically. This investigation was undertaken to compare and rank the cytotoxicity of the most commonly used ocular local anesthetics, tetracaine, proparacaine and cocaine, with primary cultures of rabbit corneal epithelial cells. Cultures were exposed to either low concentrations of local anesthetics for 4-24 h or to higher concentrations of local anesthetics for 15-120 min. Plasma membrane integrity was evaluated by measuring leakage of the cytosolic enzyme, lactate dehydrogenase, into the medium. Cell shape changes were evaluated by observing morphol. changes. Mitochondrial dehydrogenase activity and cell viability were assessed by measuring 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide redn. The cytotoxicity of the local anesthetics as evaluated by the lactate dehydrogenase leakage cytotoxicity test was ranked as follows: tetracaine (EC50 = 0.96 mM) .mchgt. proparacaine (EC50 = 4.4 mM) > cocaine (EC50 = 9.7 mM). The mitochondrial redn. assay seemed to be more sensitive than the lactate dehydrogenase leakage test in predicting toxicity: tetracaine (EC50 = 0.81 mM) .mchgt. proparacaine (EC50 = 3.4 mM) > cocaine (EC50 = 7.1 mM). When corneal epithelial cells were treated with local anesthetics, marked morphol. changes occurred at concns. that did not cause a decrease in viability. This was esp. true for cocaine-treated cells. Tetracaine and proparacaine have the same anesthetic potency in vivo, although tetracaine is considered to be more irritating than proparacaine. This in vitro study showed that tetracaine was approx. four times more toxic than proparacaine. Cocaine was less toxic in vitro than proparacaine and tetracaine when compared on an equimolar basis, but in vivo it may be more toxic because of the higher concns. that must be used to obtain the same degree of anesthesia as well as its marked effects on cell morphol.

57
Ricker HA, Neal PJ, Kubilus J, Klausner M. INITIAL CHARACTERIZATION OF A NEW MODEL FOR OCULAR IRRITANCY TESTING. Meeting of the Tissue Culture Association on Regulation of Cell and Tissue Differentiation, Research Triangle Park, North Carolina, USA, June 4-7, 1994. In Vitro Cellular & Developmental Biology Animal 1994;30A(3 Part 2):84.

No abstract.

ORAL TOXICITY

58

Polyzois GL. IN VITRO EVALUATION OF DENTAL MATERIALS. Clin Mater 1994;16(1):21-60.

Biocompatibility has been described as the ability of a material to perform with an appropriate host response in a specific application. Appropriate host response means no (or a tolerable) adverse reaction of a living system to the presence of such a material. An adverse reaction may be due to the toxicity of a dental material. Therefore toxicity may be regarded as one reason for nonbiocompatibility of a dental material. The toxicity of a dental material can be evaluated by in vitro tests, animal experiments and clinical trials. There exists a variety of different in vitro tests methods. The most widely used biological systems for toxicity screening of dental materials are cell cultures. Cell cultures for toxicity screening of dental materials are valuable tools for understanding their biological behavior, if the limitations of the methods are taken into consideration, especially concerning the interpretation of the results. Further research should concentrate on better simulations of the in vivo situation in cell cultures. In this review the applications of various cell culture methods to evaluate the cytotoxicity of a wide range of dental materials, e.g. metals, alloys, polymers and cements, are described.

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Donnelly T, Decker B, Stemp M, Rheins LA, Logemann P. ASSESSMENT OF THE OCULAR IRRITANCY POTENTIAL OF HAIR CONDITIONERS IN A THREE-DIMENSIONAL IN VITRO HUMAN SKIN MODEL. J Toxicol Cutaneous Ocul Toxiocl 1994;13(2):117-125.

A three-dimensional human tissue model has been developed in our laboratory and used as a substrate for assessing ocular cytotoxicity and irritancy potential. The full-thickness model is a coculture of dermal fibroblasts and epithelial cells (keratinocytes). Metabolically and mitotically active fibroblasts are seeded onto medical-grade nylon mesh, where they attach and secrete collagen and extracellular matrix proteins. Then keratinocytes are seeded onto the top of this submerged stromal tissue, and the tissue grows into a multilayered epithelium. Histologic examination of this cellular coculture system reveals a cellular organization similar to the rabbit/human cornea. The resulting substrate has been used effectively to study the effects of a variety of test compounds (surfactants, powders, creams) at diluted or full-strength concentrations in a time-course assay. The protocol mimics the way in which in vivo animal testing (Draize eye testing) is performed. The treated tissue is assayed for cytotoxicity (using MTT) over a period of

time. The high correlation (r = 0.87) of our in vitro data with existing animal eye data shows the potential usefulness of these tissue substrates as an in vitro alternative for evaluating the toxicity of hair care formulations.

RESPIRATORY TOXICITY

60

Knebel JW, Aufderheide M, Emura M. CHARACTERIZATION OF HUMAN BRONCHIAL CELL LINES AND EVALUATION OF THEIR METABOLIC CAPACITY FOR CHEMICAL PRECARCINOGENS. Annual Meeting of the Deutsche Gesellschaft Fuer Zellbiologie (German Society for Cell Biology), Luebeck, Germany, March 20-24, 1994. European Journal of Cell Biology 1994;63(Suppl 40):69.

No abstract.

61

Katsnelson BA, Konyscheva LK, YE Sharapova N, Privalova LI. PREDICTION OF THE COMPARATIVE INTENSITY OF PNEUMOCONIOTIC CHANGES CAUSED BY CHRONIC INHALATION EXPOSURE TO DUSTS OF DIFFERENT CYTOTOXICITY BY MEANS OF A MATHEMATICAL MODEL. Occupational and Environmental Medicine 1994;51(3):173-180.

A multicompartmental mathematical model has been used to simulate variations in the cytotoxicity of dusts in the kinetics of the retention, in the pulmonary region and tracheobronchial lymph nodes, of practically insoluble quartzite and titanium dioxide dust particles deposited on the free surfaces of the acini from alveolar air. Experiments with these dusts were conducted on rats exposed to virtually the same dust concentrations in the air for an experimental period of 20 weeks and a period of 10 weeks after exposure. Satisfactory approximation to the experimental data on the retention of these dusts is obtained by using the model parameters that depend either on damage to lung macrophages by phagocytosed particles or on the response of the host organism to this damage by enhanced recruitment of neutrophilic leukocytes; all the other variables of the model being unchanged. The values of the "action integral" computed from this model and multiplied by the index of comparative cytotoxicity of particles in vitro satisfactorily approximate to quantitative differences in the intensity of pneumoconioses caused by the dusts under study by the end of the experimental period. On the whole, the results of the mathematical model agree with the hypothesis that the cytotoxicity of particles plays a key part in both the process of retention of dust in the lung parenchyma and lung associated lymph nodes, and the pathological process caused by the retained dust. Thus given the factors and conditions on which the deposition of practically insoluble dusts in the pulmonary region depends, it is necessary to take into account the multiplicative

nature of these two effects of cytotoxicity when predicting the comparative risk of pneumoconiosis.

SKIN/DERMAL TOXICITY

62

Harvell JD, Tsai Y-C, Maibach HI, Gay R, Gordon VC, Miller K, Mun GC. AN IN VIVO CORRELATION WITH THREE IN VITRO ASSAYS TO ASSESS SKIN IRRITATION POTENTIAL. J Toxicol Cutaneous, Ocul Toxicol 1994;13(2):171-183.

We have tested the irritancy of 10 materials of various chemical composition in three in vitro toxicity assays: the Skintex Dermal Assay system (In Vitro International, Irvine, CA, USA), the silicon microphysiometer (Molecular Devices Inc., Menlo Park, CA), and the Living Skin Equivalent (Organogenesis Inc., Cambridge, MA). The purpose was to discover to what degree the in vitro results predict in vivo skin irritation as seen in nine female volunteers over the course of a 5 day cumulative irritancy patch test. Two in vivo assessments of irritancy were made and compared to the in vitro results, a visual scoring system, and a potentially more objective bioengineering assessment; the chromameter. Measures of sensitivity, specificity, positive predictive value, and negative predictive value were used to compare in vitro with in vivo results. Comparison of results using correlation coefficients was avoided since statistically significant rank orders could not be demonstrated for either in vitro or in vivo data. For these in vitro systems, sensitivity ranged from 60 to 100%, specificity from 80 to 100%, positive predictive value from 75 to 100%, and negative predictive value from 67 to 100%. The assay that exhibited the best measures of sensitivity, specificity, and other parameters was the Living Skin Equivalent. This assay is also the one that most closely simulates human skin. The nonionic surfactant triton X-100 gave a false-positive result in both the Living Skin Equivalent and the silicon microphysiometer. False-positive results with nonionic surfactants are consistent with observations made in other cell-based in vitro systems. We caution that the results not be over-interpreted to imply superiority for any one system.

63

Kreppel H, Reichl FX, Knopff G, Peter R, Szinicz L. EFFECT OF ARSENICALS ON SKIN IN VITRO. 35th Spring Meeting of the German Society for Experimental and Clinical Pharmacology and Toxicology, Mainz, Germany, March 15-17, 1994. Naunyn-Schmiedeberg's Archives of Pharmacology 1994; 349(Suppl):R117.

No abstract.

64
Spielmann H, Balls M, Holzhuetter HG, Kalweit S, Klecak G, Liebsch M, L'eplattenier H, Lovell W, Maurer T, Moldenhauer F, Moore L, Pape W, Pfannbecker U, Potthast J, De Silva O, Steiling W, Willshaw A. EEC-COLIPA PROJECT ON IN VITRO PHOTOIRRITANCY TESTING. 35th Sprign Meeting of the German Society for Experimental and Clinical Pharmacology and Toxicology, Mainz, Germany, March 15-17, 1994. Naunyn-Schmiedeberg's Archives of Pharmacology 1994;349(Suppl): R108.

No abstract.

65

Kietzmann M, Maass P, Loescher W. THE ISOLATED PERFUSED BOVINE UDDER AS A MODEL FOR SKIN IRRITATION TESTS? 35th Spring Meeting of the German Society for Experimental and Clinical Pharmacology and Toxicology, Mainz, Germany, March 15-17, 1994. Naunyn-Schmiedeberg's Archives of Pharmacology 1994; 349(Suppl):R108.

No abstract.

66
Roguet R, Cohen C, Dossou KG, Rougier A. EPISKIN, A RECONSTITUTED HUMAN EPIDERMIS FOR ASSESSING IN VITRO THE IRRITANCY OF TOPICALLY APPLIED COMPOUNDS. Toxicol In Vitro 1994;8(2):283-291.

The Episkin epidermal system is a reconstituted organotypic culture of human keratinocytes forming a multilayer differentiated epidermis on a collagen matrix. This standardized model was used to measure various parameters reflecting irritation observed in vivo following topical application of a variety of surfactants. A comparison of the results with historical data from ocular irritation testing in vivo showed a strong correlation for cytotoxic potential ($r = 0.93$; $n = 23$; $P < 0.00001$) and damage to the epithelial barrier function ($r = 0.87$; $n = 20$; $P < 0.00001$). In addition, the proportion of agents for which the irritative potential is underestimated is low. With regard to cutaneous irritation, the release of a major pro-inflammatory cytokine, interleukin 1alpha, correlated with the degree of irritation observed in vivo ($r = 0.81$; $n = 20$; $P < 0.0001$). Given its suitability to testing a wide variety of parameters reflecting irritation and the fact that all types of topical formulations can be applied to the reconstructed epidermal surface, the Episkin model should be particularly

useful for assessing the tolerability of topical agents in vitro.

67
Kotani M, Masamoto Y, Watanabe M. AN ALTERNATIVE STUDY OF THE SKIN IRRITANT EFFECT OF AN HOMOLOGOUS SERIES OF SURFACTANTS. Toxicol In Vitro 1994;8(2):229-233.

An attempt has been made to differentiate between in vivo and in vitro skin reactions to a homologous series of surfactants (sodium alkyl sulfate, R-OSO3 Na) and to determine the usefulness of percutaneous absorption in vitro as an alternative test system. Sodium alkyl sulfate showed considerable biological activity by virtue of its polar head groups. The length of the lipophilic chain in the surfactants was an important factor in their overall activity. The following in vivo tests were performed: a primary skin irritation test in guinea pigs, a primary eye irritation test in rabbits and a closed patch test in humans. Peak skin irritation occurred with C10-C16 sodium alkyl sulfate, which had lipophilic groups of different alkyl chain lengths. Cell injury was also evaluated by the neutral red dye uptake assay in rabbit corneal (RC) cells. C4 and C6 compounds had no effect, while maximal effects occurred with C18. Protein denaturation and hemolysis occurred with C10-C16 compounds. In the percutaneous absorption test in guinea pig skin, permeation was low for the C18 compound and high for the C4 compound. The results with the C18 compound suggest that differences between cell injury and skin irritation result from skin permeation. Although the C18 compound caused cell injury, membrane destruction and protein denaturation were more severely with the C10-C16 compounds, owing to their strong haemolytic and protein-denaturation action.

68
Hughes MF, Fisher HL, Birnbaum LS, Hall LL. EFFECT OF AGE ON THE IN VITRO PERCUTANEOUS ABSORPTION OF PHENOLS IN MICE. Toxicol In Vitro 1994;8(2):221-227.

The effect of age on the in vitro dermal absorption of acetamidophenol, phenol, cyanophenol and heptyloxyphenol was examined. Skin from pre-clipped male C57BL/6N mice of ages 3, 15 and 27 months was mounted in flow-through diffusion cells. (14C)Phenol and its analogues (4 mug/cm2) were applied to the skin (0.32 cm2) in ethanol (5 mul) and absorption was measured under occluded conditions for 72 hr. Significant age effects on the disposition of phenol and heptyloxyphenol were observed in the penetration of compound into the receptor fluid as well as that retained in the skin. The receptor fluid content of these two compounds was significantly greater in 27-month-old mice compared with the younger animals. In addition, penetration

of phenol in 15-month-old mice was significantly greater than in 3-month-old mice. However, the differences in phenol penetration were small (< 5%). The maximal flux of cyanophenol and phenol penetration were affected by age. The maximal flux for cyanophenol was significantly lower in 15-month-old mice than in 3- and 27-month-old animals. The maximal flux for phenol was significantly greater in 27-month-old mice compared with the younger animals. The overall effect of age on the in vitro dermal absorption of phenols in C57BL/6N mouse skin appears to be compound dependent.

69
Korting HC, Schindler S, Hartinger A, Kerscher M, Angerpointner T, Maibach HI. MTT-ASSAY AND NEUTRAL RED RELEASE (NRR)-ASSAY: RELATIVE ROLE IN THE PREDICTION OF THE IRRITANCY POTENTIAL OF SURFACTANTS. Life Sci 1994;55(7):533-40.

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

70
Jacobs RR, Phanprasit W. AN IN VITRO COMPARISON OF THE PERMEATION OF CHEMICALS IN VAPOR AND LIQUID PHASE THROUGH PIG SKIN. American Indus Hygiene Assn J 1993;54(10):569-575. (18 REFS)

Pig skin was used to compare vapour and liquid permeation of benzene, n-butanol, and toluene in vitro. Vapours of radio-labelled chemicals were generated by passing purified air through two saturators in series containing the labelled chemical. The generated vapour was directed into the donor compartment of a modified liquid permeation cell. For liquid permeation experiments, neat chemicals were dosed directly on the surface of the skin. The variability of the generated concentrations for the vapour phase of each chemical ranged from 3 to 7%. The mean flux of the liquid chemicals was significantly higher than those of the vapour phase. There was no significant difference in the flux of the individual chemicals in the liquid phase. In the vapour phase test, the fluxes of toluene and benzene were not significantly different; however for n-butanol the flux was significantly lower than for either benzene or toluene.

71
Snider TH, Hobson DW. MULTI-PHASED SCREEN FOR THE EVALUATION OF TOPICAL SKIN PROTECTANTS AGAINST VARIOUS CHEMICALS. Govt Reports Announcements & Index (GRA&I), Issue 13, 1994.

A multi-phased screen involving both in vivo and in vitro tests was used to evaluate the efficacy of 108 topical skin protectants (TSPs) against dermal exposure to sulfur mustard (HD), pinacolyl methylphosphonofluoridate (soman or GD), thickened soman (TGD), and 0-ethyl S-(2-diisopropylaminoethyl) methylphosphonothioate (VX). Assessment of TSPs in vivo involved the application of chemical agents onto a 0.1 mm thickness of TSP spread on the dorsa of rabbits. For the nerve agents GD, TGD, and VX, acetylcholinesterase (AChE) inhibition in lysed red blood cells sampled periodically to 24 hr after dose application was used as an end point. Efficacy against the vesicating agent HD was assessed using the areas of dermal lesions from 1 microns L dosed at multiple sites on rabbits. The in vitro model involved delivery of 8 microns L HD or nerve agent on candidate TSPs applied at 0.015 mL/sq cm on U.S. Army M-8 chemical agent detection paper. The in vitro end point for TSP efficacy evaluation was the time to M-8 paper color change, indicating time to agent penetration. In vitro/in vivo correlations indicated good agreement for HD, GD, and TGD challenges, but not for VX. This article is from 'Proceedings of the Medical Defense Bioscience Review (1993) Held in Baltimore, Maryland on 10-13 May 1993. Volume 1', AD-A275 667, p305-312.

72
Mershon MM, Rhoads LS, Van Buskirk RG. USE OF FLUORESCENT DYES AND SPECTROFLUOROMETRY TO OBSERVE EVIDENCE OF VESICANT TOXICITY IN HUMAN EPIDERMAL CELLS. Govt Reports Announcements & Index

(GRA&I), Issue 13, 1994.

Normal human epidermal keratinocyes (NHEK) show multiple dose-related biochemical ranges at 3 hours after in vitro exposure to a vesicant compound, 2-chloroethyl ethyl sulfide (CEES) CEES in ethanol was diluted to 0.8, 8.0 and 80 mM concentrations in cell culture medium over confluent NHEK on gel-coated membranes of Millipore Millicells or in NHEK suspensions. A site-specific fluorescent dye was incubated with each NHEK layer for 1 hr prior to comparisons of normal and challenged NHEK within a Cytofluor 2300 spectrofluorometer. Reduced fluorescence from loss of all dye probes indicated severe membrane damage with 80 mM CEES in medium. Intracellular increases in Ca++, evidence of altered mitochondrial activity, and decreases in pH, glutathione levels and lysosomal integrity, were observed with 0.8 and 8.0 mM CEES in the culture medium. Control studies performed with Testskin and another human epidermal model suggest that dermal substitutes and transportation stresses can influence results with the dye probe/Cytofluor 2300 method. However, the feasibility of using the described methods to observe vesicant biochemical effects, screen antivesicants and perform other toxicological studies with NHEK models is supported by the results of the preliminary studies. This article is from 'Proceedings of the Medical Defense Bioscience Review (1993) Held in Baltimore, Maryland on 10-13 May 1993. Volume 1', AD-A275 667, p77-86.

73
Kasparkova V, Hybasek P. EFFECT OF HERBICIDES ON HUMAN SKIN: PENETRATION OF HERBICIDES THROUGH THE EPIDERMIS "IN VITRO". Acta Univ Palacki Olomuc, Fac Med 1992;133:15-18.

Penetration of herbicides Gramoxone and Reglone through human epidermis was studied in vitro. A special diffusion equipment was used and the method of A. Calderbank and S. H. Yuen (1965) was modified for quant. detn. of active components (paraquat, diquat) of both herbicides. Penetration of these herbicides was different and surfactants contained in herbicides were found to play a role in penetration into the skin.

74
Fullerton A, Broby-Johansen U, Agner T. SODIUM LAURYL SULFATE PENETRATION IN AN IN VITRO MODEL USING HUMAN SKIN. Contact Dermatitis 1994;30(4):222-5.

In the present study, penetration of S35-labeled sodium lauryl sulfate (SLS) was studied in an in vitro model using human cadaver skin. The investigations showed that SLS is capable of permeating the skin barrier when applied under occlusion. SLS

could be detected in the dermis and the amt. of SLS found here was shown to depend on the dose of SLS applied on the skin. Penetration of SLS continued after removal of the SLS applied as a patch test on the skin surface. Considerable inter-individual variation in the penetration of SLS was demonstrated between different donors.

TERATOGENICITY

75

Cumberland P FT, Richold M, Parsons JF, Pratten MK. FURTHER EVALUATION OF A TERATOGENICITY SCREEN USING AN INTRAVITELLINE INJECTION TECHNIQUE. Toxicol In Vitro 1994;8(2):153-166.

A blind trial of 31 compounds was conducted in order to evaluate the intravitelline injection technique as a predictor of developmental toxicity. Rat conceptuses were explanted on day 9 of gestation and cultured until 11.5 days, at which time the compounds were injected into the vitelline circulation of the yolk sac. The embryos were then cultured for a further 24 hr after which time they were assessed on 14 parameters and assigned to one of three categories based on predetermined criteria. The results were then compared with the classifications previously allocated using data derived from in vitro studies. The compounds were examples of non-teratogens, non-cytotoxic teratogens and cytotoxic teratogens. Of the 31 compounds, 23 gave the predicted result, three non-teratogens being false positives and five teratogens having no effect in this system. The results therefore demonstrate the ability of the technique to distinguish between the three categories of compound, displaying a specificity of 75%, a sensitivity of 74% and an overall accuracy of 74.5%. Furthermore, it allowed the differentiation of active and inactive analogues of four paired compounds. This method may have applications as a teratogenicity screen or in the elucidation of the mechanism of action of teratogenic compounds.

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Cumberland PF T, Richold M, Parsons JF, Pratten MK. FURTHER EVALUATION OF A TERATOGENICITY SCREEN USING AN INTRAVITELLINE INJECTION TECHNIQUE. Toxicol in Vitro 1994;8(2):153-66.

A blind trial of 31 compounds was conducted in order to evaluate the intravitelline injection technique as a predictor of developmental toxicity. Rat conceptuses were explanted on day 9 of gestation and cultured until 11.5 days, at which time the compds. were injected into the vitelline circulation of the yolk sac. The embryos were then cultured for a further 24 h after which time they were assessed on 14 parameters and assigned to one of three categories based on predetd. criteria. The results

were then compared with the classifications previously allocated using data derived from in vitro studies. The compounds were examples of non-teratogens, non-cytotoxic teratogens and cytotoxic teratogens. Of the 31 compounds, 23 gave the predicted result, three non-teratogens being false positives and five teratogens having no effect in this system. The results therefore demonstrate the ability of the technique to distinguish between the three categories of compd., displaying a specificity of 75%, a sensitivity of 74% and an overall accuracy of 74.5%. Furthermore, it allowed the differentiation of active and inactive analogs of four paired compds. This method may have applications as a teratogenicity screen or in the elucidation of the mechanism of action of teratogenic compounds.

VALIDATION/STANDARDIZATION TESTS

77

Schmid B, Zbinden G. BIOLOGICAL ASSESSMENT OF CHEMICAL DNA DAMAGE IN GERM CELLS OF MALE RABBITS. Methods Find Exp Clin Pharmacol 1980;2(6):319-26.

DNA damage represents a key step in mutagenesis and carcinogenesis. Excision repair has been described to be the major pathway restoring damaged DNA. Assaying DNA repair may thus illuminate the mechanism of mutagenesis and carcinogenesis and serve as an indicator of the mutagenic and carcinogenic potential of environmental chemicals and drugs. The paper demonstrates a practical application of these concepts. DNA damage induced by chemical substances in male germ cells of rabbits was assessed by the demonstration of DNA repair synthesis in meiotic and post-meiotic maturation stages. Incorporation of tritium-labeled thymidine was monitored in spermatozoa obtained by serial ejaculation. The test was validated with several standard mutagens and carcinogens, and its usefulness was demonstrated with a study on 3 suspected genotoxic drugs, i.e. hycanthone, isoniazid, and metronidazole.

78

Morita T, Kondo K. CURRENT STATUS AND PROBLEMS OF INTERNATIONAL STANDARDIZATION ON THE PROCEDURE OF IN VITRO CHROMOSOMAL ABERRATION TEST. Kankyo Hen'igen Kenkyu 1994; 16(1):141-50. (14 REFS)

International standardization of in vitro chromosomal aberration test procedures was discussed in the international workshop in Melbourne. The upper limit of testing should be 10 mM or 5 mg/mL, whichever is lower. Cytotoxicity at the top dose should be greater than 50% of concurrent negative control, if this can be achieved without exceeding a concn. limit of 10 mM or 5 mg/mL. It was not possible to reach a consensus on the issue of soly.

limits. However, it was acceptable to include one top dose level with evident ppt. as a pragmatic way of demonstrating that the soly. limit in the cultures had been achieved. Treatment length both with and without S9 should be for 3 to 6 h, followed by sampling at a time equiv. to about 1.5 normal cell cycle (NCC) lengths from the beginning of treatment. If this protocol gives negative results both with and without S9, an addnl. test without S9 should be done, with continuous treatment for 1.5 NCC lengths. Many items were discussed in addition to the above issues, and those were reached consensus.

79
Taniguchi Y, Suzuki K, Nakajima K, Nakajima M, Miwa Y, Yamada Y, Satoh M, Takeyoshi M, Akie Y, et al. INTER-LABORATORY VALIDATION STUDY OF THE SKIN2 DERMAL MODEL ZK1100 AND MTT CYTOTOXICITY ASSAY KITS. J Toxicol Sci 1994;19(1):37-44.

An inter-lab. validation study was conducted to evaluate the potential of 4 chemicals to cause irritation utilizing the Skin2 Dermal Model ZK1100 kit developed by Advanced Tissue Sciences, Inc. (formerly Marrow-Tech, Inc., La Jolla, California, USA). The chemicals tested were sodium dodecyl sulfate (SDS), 1-n-hexadecylpyridinium chloride monohydrate (CC), ethanol (EtOH), and DMSO. Eleven Japanese institutions participated in this validation research to evaluate the usefulness of the Skin2 Model ZK1100 kit in accordance with an identical protocol. None of the participating labs. had previously used the Skin2 Model ZK1100 kit. The MTT-50 value obtained in the individual institutions was 42 to 91 mug/mL for SDS, 2.7 to 8.6 mug/mL for CC, 2.0 to 9.3% for EtOH, and 11.5 to 21.9% for DMSO. Reproducibility was reasonably good as noted when one test chem. was repetitively tested by the same investigator. MTT-50 values obtained with the present method correlated with DS20 values obtained with Draize's method (r=0.9881) in one of the participant institutions. The irritation study using the Skin2 Model ZK1100 kit was easy to perform and generated quant. data. When the test was repeated, reproducibility was demonstrated with a variation of less than 2 sigma. These data suggested that this newly developed in vitro method would be useful in toxicity screening studies in terms of both time and cost, and would serve as a useful alternative to the conventional methods of the eye irritation study.

XYZ/MISCELLANEOUS
80
Kirkland DJ. REPORT OF THE IN VITRO SUB-GROUP. Mutat Res 1994;312(3):211-215.

No abstract.

81
Flaig KH, Elsenhans B, Schuemann K, Forth W. RAT INTESTINAL ABSORPTION OF CADMIUM IN VIVO AND IN VITRO A COMPARISON. 35th Spring Meeting of the German Society for Experimental and Clinical Pharmacology and Toxicology, Mainz, Germany, March 15-17, 1994. Naunyn-Schmiedeberg's Archives of Pharmacology 1994;349(Suppl):R117.

No abstract.

82
Warith MA, Yong RN. TOXICITY ASSESSMENT OF SLUDGE FLUID ASSOCIATED WITH TARS AND TAILINGS. Environ Technol 1994; 15(4):381-387.

This study was conducted in an effort to assess the toxicity of fluid emanating from potential sludges produced as a result of the "hot water extraction process" employed in extracting oil from tar sand deposits in Alberta. A further attempt was made to identify specific components and/or properties which might be responsible for any toxicity observed in the emanating sludge fluid. Fluids emanating from 14 different potential sludges resulting from various proposed treatment processes were considered. All emanating fluids were observed to be highly toxic to the green algae, Selenastrum capricornutum, with an average lethal concentration (LC-50) of 25.8% (by volume).

83
Fischer EG. THE 24-WELL PLATE CELL MIGRATION ASSAY. Annual Meeting of the Deutsche Gesellschaft Fuer Zellbiologie (German Society for Cell Biology), Luebeck, Germany, March 20-24, 1994. European Journal of Cell Biology 1994;63(Suppl 40):11.

No abstract.