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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.

10
Fritzenschaf H, Kohlpoth M, Rusche B, Schiffmann D.
TESTING OF KNOWN CARCINOGENS AND NONCARCINOGENS IN THE SYRIAN HAMSTER EMBRYO (SHE) MICRONUCLEUS TEST IN VITRO; CORRELATIONS WITH IN VIVO MICRONUCLEUS FORMATION AND CELL TRANSFORMATION. Mutat Res 1993;319(1):47-53.

Seventy-five chemicals, carcinogens and noncarcinogens, were tested in the SHE (Syrian hamster embryo) micronucleus test in vitro. Substances inducing a reproducible and dose dependent increase in micronucleus frequency were regarded as positive. The acquired data were analyzed for correlations with results obtained from the in vivo mouse bone marrow micronucleus test and from morphological transformation of SHE cells. Out of 48 carcinogens tested 41 (85%) yielded a positive result and out of 17 noncarcinogens all proved negative. For 7 chemicals no carcinogenicity data were available so far; these compounds yielded no response in the mouse bone marrow and in the SHE micronucleus assay. For 3 chemicals only inadequate carcinogenicity data were available. A high degree of concordance with data from the in vivo micronucleus test was found (89%) and the accordance with results from morphological SHE cell transformation was even higher (95%). These findings provide new evidence that the in vitro SHE micronucleus test does in fact represent a short-term test of high predictive value.

11
Brill S, Holst P, Sigal S, Zvibel I, Fiorino A, Ochs A, Somasundaran U, Reid LM. HEPATIC PROGENITOR POPULATIONS IN EMBRYONIC, NEONATAL, AND ADULT LIVER. Proc Soc Exp Biol Med 1993; 204(3):261-9. (REFS: 26)

Oval cells, small cells with oval-shaped nuclei, are induced to proliferate in the livers of animals treated with carcinogens and are thought to be related to liver stem cells and/or committed liver progenitor cell populations. We have developed protocols for identifying and isolating antigenically related cell populations present in normal tissues using monoclonal antibodies to oval cell antigens and fluorescence-activated cell sorting. We have isolated oval cell-antigen-positive (OCAP) cells from embryonic, neonatal, and adult rat livers and have identified culture conditions permitting their growth in culture. The requirements for growth of the OCAP cells included

applicability of this test in primary cultures of hepatocytes. The induction of micronuclei (MN) by methyl methanesulphonate (2 mM) and by the indirect carcinogens cyclophosphamide (CP, 0.4-4 mM) and diethylnitrosamine (DEN, 1-10 mM) has therefore been studied in rat liver cells in vitro. Analysis and quantification of MN, as well as determination of the proliferative activity of the hepatocytes, was performed by flow cytometric techniques. All three chemicals increased the frequency of MN at incubation times of more than 48 hr. The relative increase in MN compared with that in untreated or solvent-treated cultures, however, was at the most only three-fold, since the frequency of MN increased markedly in the control cultures also. There was a marked decrease in proliferative activity of the hepatocytes, as shown by the decrease in frequency of cells in the second G1-phase at the highest concentration of CP and at all concentrations of DEN. In conclusion, flow cytometric analysis of MN enables a fast and reliable determination of cytogenetic effects in hepatocyte cultures treated with chemicals, However, the large number of MN in untreated hepatocytes, which is possibly a consequence of DNA damage induced by the isolation procedure, may limit the sensitivity of the method.

CARDIOTOXICITY

13

Weisensee D, Low-Friedrich I, Riehle M, Bereiter-Hahn J, Schoeppe W. IN VITRO APPROACH TO 'UREMIC CARDIOMYOPATHY'. Nephron 1993; 65(3):392-400.

Cardiovascular complications determine the prognosis of patients with chronic renal failure. The contribution of compounds retained during uremia to specific myocardial lesions is controversial. We investigated the contractility of spontaneously beating mouse cardiac myocytes in culture under perfusion with sera derived from patients on maintenance hemodialysis and test solutions containing possible toxins. Cellular contractility under defined environmental conditions is determined by a computer-assisted digital image analysis. 'Uremic sera', creatinine, urea, and combinations of these compounds reduce inotropy of the cultured heart cells, induce arrhythmias or asynchronies in a concentration-dependent manner. We propose the myocyte perfusion technique as an in vitro approach to identify cardiotoxins in the body fluids of chronically uremic patients.

been passaged approximately 100 times over an 8-year period. The cells do not form colonies or grown in soft agar. The cultures are heteroploid. The cell shape was predominantly polygonal or epithelial-like, but as cultures became confluent, bipolar or fibroblast-like cells appeared. Among the prominent ultrastructural features of RTL-W1 were distended endoplasmic reticulum and desmosomes. Benzo(a)pyrene was cytotoxic to RTL-W1. Activity for the enzyme, 7-ethoxyresorufin O-deethylase (EROD), which is a measure of the cytochrome P4501A1 protein, increased dramatically in RTL-W1 upon their exposure to increasing concentrations of either beta-naphthoflavone (BNF) or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). With these properties, RTL-W1 should be useful for studying the expression of the cytochrome P450 enzymes and as a tool for assessing the toxic potency of environmental contaminants.

19
Meyers U, Szulczewski DH, Barckhaus RH, Atkinson M, Jones DB. BIOLOGICAL EVALUATION OF AN IONOMERIC BONE CEMENT BY OSTEOBLAST CELL CULTURE METHODS. Biomaterials 1993; 14 (12): 917-924.

Periosteal derived bovine osteoblast-like cells migrated in culture onto an ionomeric cement. Cell cultures were maintained for 4 weeks and used to study the in vitro behaviour of cells on the ionomeric bone cement (IC). The cells produced bone matrix proteins (osteocalcin, bone sialoprotein II) and were osteoblast-like. The osteoblast-like cells colonized the substrate in monolayers and produced an extracellular matrix as seen by light and scanning electron microscopy. Morphological comparison between cells growing on the ionomeric bone cement and cortical bone revealed no significant difference in phenotypic expression. Staining for aluminium in osteoblasts growing on the IC showed an uptake and storage of aluminium in the cells. Energy dispersive X-ray microanalysis revealed high concentrations of aluminium and silicon in the periosteal tissue. Despite the known toxic effect of aluminium in vivo and in vitro on osteoblasts, no signs of toxicity were apparent on light and scanning electron microscopy analysis.

20
Li AP. PRIMARY HEPATOCYTE CULTURE AS AN IN VITRO TOXICOLOGICAL SYSTEM OF THE LIVER Gad, S. C. (ED.). In Vitro Toxicology. IX+290P. Raven Press: New York,

New York, USA. ISBN 0-88167-974-7.; 0 (0). 1994. 195-220.

No abstract.

21
Miranda CL, Collodi P, Zhao X, Barnes DW, Buhler DR. REGULATION OF CYTOCHROME P450 EXPRESSION IN A NOVEL LIVER CELL LINE FROM ZEBRAFISH (BRACHYDANIO RERIO). Arch Biochem Biophys 1993;305(2):320-327.

The expression and induction of cytochrome P450 by 2,3,7,8-tetrachlordibenzo-p-dioxin (TCDD) and beta-naphthoflavone (BNF)in a new liver cell line from adult zebrafish (Brachydanio rerio) were studied. Subcellular fractions from control, BNF- or TCDD-treated cells did not show detectable bands in immunoblots probed with antibodies to the constitutive forms of trout P450 (LMC1, LMC2, LMC3, LMC4, and LMC5), suggesting that either zebrafish liver cells lack P450s closely related to those constitutively expressed in trout or that the concentrations of the orthologous P450s were too low to be detected. However, upon exposure to TCDD, the cells expressed a major immunoreactive 54-kDa protein and a minor 50-kDa protein recognized by antibodies to rainbow trout P4501A1. These immunoreactive proteins were observed in microsomal and mitochondrial fractions of TCDD-treated cells but were not detected in cell cultures treated with dimethyl sulfoxide (DMSO) (vehicle control) or BNF. The activities of ethoxyresorufin beta-deethylase (EROD) and 7,12- dimethylbenzanthracene (DMBA) hydroxylase were markedly increased by TCDD but not by BNF in this cell line. EROD activity was more sensitive than DMBA hydroxylase activity of TCDDtreated liver cells to diagnostic inhibitors such as alphanaphthoflavone and anti-trout P4501A1 IgG. The TCDD-treated cells converted DMBA to various metabolites, one of which is the putative proximate carcinogen, DMBA-3,4-diol. These results suggest that TCD but not BNF, induces one or possibly two forms of P450 immunochemically and functionally related to trout P4501A1,in cultured zebra fish liver cells.

22
Tiffany-Castiglioni E. CELL CULTURE MODELS FOR LEAD TOXICITY IN NEURONAL AND GLIAL CELLS. Neurotoxicology (Little Rock)1993;14(4):513-536.

formazan assay the authors found comparable IC50 values but this new assay was more economical and results are obtained in two hours as compared to four hours for the formazan assay. This new economical and versatile assay could be used with advantage for large scale in vitro screening of anticancer drugs and other cytotoxic agents.

38
Segner H, Lenz D. CYTOTOXICITY ASSAYS WITH THE RAINBOW TROUT R1 CELL LINE. Toxicol in Vitro 1993;7(4):537-40.

In this study, cells of the fibroblast-like R1cell line from rainbow trout (Oncorhynchus mykiss) were exposed to a total of 30 org. and inorg. chems., in order to reveal possible correlations between R1 cytotoxicity and (a) toxicity to fish in vivo, (b) cytotoxicity data from other established fish cell lines, and (c) physicochem. parameters of the test agents. Cytotoxicity was assessed using the crystal violet (CV) staining technique, which dets. the no. of cells that are able to attach to the culture substratum during a 24-h exposure period. For a broad spectrum of test compds., including heavy metals as well as org. chems., there was good correlation of in vivo lethality and in vitro cytotoxicity (n = 21, r = 0.84). However, of 21 chems., six ($HgCl_2$, $CuSO_4$, 2,4-dichlorophenol, 4-chloroaniline, chlorobenzene and phenol), were toxic to fish at concns. at least 10 times lower than those in the cytotoxicity assay, whereas the in vitro assay was found to be more sensitive than the in vivo test for one chem. only (trichloroacetic acid). R1 midpoint toxicity values (CV50), on the other hand, closely agreed with cytotoxicity data reported for other fish cell lines (FHM, BF-2, GFS), both in abs. sensitivity and in relative ranking. For the 18 org. chems. tested, a correlation between cytotoxicity in R1 cells and their octanol/water partition coeff. could be established. In conclusion, the R1 cell line seems to be a useful tool for screening studies in order to rank the toxicity of environmental pollutants or to evaluate structure-activity relationships.

39
Pizao PE, Peters GJ, Van Ark-Otte J, Smets LA, Smitskamp-Wilms E, Winograd B, Pinedo HM, Giaccone G. CYTOTOXIC EFFECTS OF ANTICANCER AGENTS ON SUBCONFLUENT AND MULTILAYERED POSTCONFLUENT CULTURES. Eur J Cancer, Part A 1993;29A(11):1566-73.

The cytotoxic effects of conventional (doxorubicin, 5-fluorouracil, cisplatin) and investigational (2',2'-difluorodeoxycytidine, hexadecylphosphocholine, EO9, rhizoxin) anticancer drugs were studied in subconfluent and multilayered postconfluent cultures of human colon and ovarian carcinoma cell lines. Chemosensitivity was assessed 4 days after a 24-h drug exposure with the sulforhodamine B assay. Except for rhizoxin, all drugs tested yielded an EC50 (drug concn. producing absorbance readings 50% lower than those of non-treated wells) in postconfluent cultures that were higher than an EC50 obtained with subconfluent cultures .Compared with subconfluent cultures, postconfluent cultures showed decreased cellular nucleotide concns. and ATP/ADP ratios, in addn. to an increased percentage of G0/G1 cells. The activity of DT-diaphorase, a reductase involved in the bioactivation of EO9, was similar in sub- and postconfluent cultures. These results indicate similarity of the postconfluent model presented with those obtained with in vivo models and more complex in vitro techniques.

40
Calleja MC, Persoone G, Geladi P. COMPARATIVE ACUTE TOXICITY OF THE FIRST 50 MULTICENTER EVALUATION OF IN VITRO CYTOTOXICITY CHEMICALS TO AQUATIC NON-VERTEBRATES. Arch Environ Contam Toxicol 1994;26(1):69-78.

The acute toxicity data of the 1st 50 chems. of the Multicenter Evaluation of In vitro Cytotoxicity (MEIC) program is compared for 3 cyst-based toxicity tests (Artoxkit M with Artemia salina, Streptoxkit F with Streptocephalus proboscideus, and Rotoxkit F with Brachionus calyciflorus), and 2 other tests (the Daphnia magna and the Photobacterium phosphoreum Microtox tests) commonly used in ecotoxicol. The difference in sensitivity for the 50 chems. was as high as 19 orders of magnitude (on a mol. wt. basis) between the most and least sensitive species. Generally, a similar toxicity ranking of the 5 test species was found for most of the chems. and the interspecies correlations were high. Results from Principal Components Anal. (PCA) and cluster anal. indicated that the groupings are not related to a clear and defined chem. structure. However, the loading plot of the 1st 2 principal components may aid in selecting the min. no.

In vitro screening of a no. of 2-(aminoalkyl)-5-nitropyrazolo[3,4,5-kl]acridines has previously indicated (Sebolt, et al. 1987) that these compounds, in general, exhibit selective cytotoxicity against the human colon adenocarcinoma, HCT-8, cell line, relative to mouse leukemia L1210 cells. Comparative mol. field anal. (CoMFA) was applied to HCT-8 and L1210 growth inhibition assays (IC50s) of a series (44) of the pyrazoloacridine derivs. with the objective of predicting improved solid tumor selectivity. In the absence of crystallog. data, the 9-methoxy deriv., which is currently in clin. study, was selected as the template mol. model. Two different structural alignments were tested: an alignment of structures based on root mean square (RMS)-fitting of each structure to the 9-methoxy deriv. was compared with an alternative strategy, steric and electrostatic alignment (SEAL). Somewhat better predictive cross-validation correlations (r2) were obtained with models based on RMS vis-a-vis SEAL alignment for both sets of assays. A large change in lattice spacing, e.g., 2 to 1 .ANG., causes significant variations in the CoMFA results. A shift in the lattice of half of its spacing had a much smaller effect on the CoMFA data for a lattice of 1 .ANG. than one of 2 .ANG.. The relative contribution of steric and electrostatic fields to both models were about equal, underscoring the importance of both terms. Neither calcd. log P nor HOMO and/or LUMO energies contribute to the model. Steric and electrostatic fields of the pyrazoloacridines are the sole relevant descriptors to the structure-activity (cross-validated and conventional) correlations obtained with the cytotoxic data for both the L1210 and HCT-8 cell lines. The cross-validated r2, derived from partial least-squares calcns., indicated considerable predictive capacity for growth inhibition of both the leukemia and solid-tumor data. Evidence for the predictive performance of the CoMFA-derived models is provided in the form of plots of actual vs predicted growth inhibition of L1210 and HCT-8 cells, resp., by the pyrazoloacridines. The steric and electrostatic features of the QSAR are presented in the form of std. deviation coeff. contour maps of steric and electrostatic fields. The maps indicate that increases or decreases in steric bulk that would enhance growth inhibition of HCT-8 cells would likewise promote growth inhibition of L1210 cells. Contour maps generated to analyze the electrostatic field contributions of the pyrazoloacridines to growth inhibition provided an

essentially similar set of results. It is apparent that steric and electrostatic fields alone are inadequate in the CoMFA to characterize the in vitro solid tumor selectivity of the pyrazoloacridines. This points to a need to supplement the cytotoxic data with results of further study that focuses on a quant. comparison of the potential for differential metabolic activation of the pyrazoloacridine.

46
Ciapetti G, Stea S, Cenni E, Sudanese A, Marraro D, Toni A, Pizzoferrato A. TOXICITY OF CYANOACRYLATES IN VITRO USING EXTRACT DILUTION ASSAY ON CELL CULTURES. Biomaterials 1994; 15(2):92-6.

Comparative cytotoxicity testing of four cyanoacrylate adhesives suggested for orthopedic applications was performed. These substances were placed in complete culture medium with serum and the resulting extn. fluids were tested on L 929 cells and human lymphocytes. Testing procedures include cell morphol. assessment using light microscopy and vital dyes, cell counting using a computer-assisted image anal. system, cell growth measurement using total protein content assay and cell viability assessment using the MTT method. Quantitation of the toxicity of the degrdn. products released by cyanoacrylates in the exts. was achieved and differences in the cytopathic effect related to the chem. compn. of the cyanoacrylates were found. A toxicity rating of the assayed cyanoacrylate adhesives was obtained.

47
Popper HH, Grygar E, Ingolic E, Wawschinek O. CYTOTOXICITY OF CHROMIUM-III AND -VI COMPOUNDS. I. IN VITRO STUDIES USING DIFFERENT CELL CULTURE SYSTEMS. Inhalation Toxicol 1993;5(4):345-369.

The cytotoxicity of trivalent-chromium (Cr+3) and hexavalent- chromium (Cr+6) compounds was studied in various cell culture systems. Chinese-hamster V79 cells, rat type-II pneumocytes (LEC cells), human adenocarcinoma (A549) cells, or guinea-pig alveolar macrophage cultures were incubated with 0 to 24,668.455 micromolar (microM) chromic-chloride, chromium-trioxide, barium- chromate, lead-chromate, or potassium-dichromate for up to 18 hours. Cytotoxicity was assessed by measuring the effects on colony forming ability and survival by the trypan-blue dye test. Cr+6

and Cr+3 uptake by V79 and LEC cells was determined by measuring the chromium content of the cells. The alveolar macrophages were examined for histomorphological changes. The supernatant from alveolar macrophages treated with potassium-dichromate was added to guinea-pig fibroblasts for 2 or 12 hours. The effects on fibroblast growth and mitosis were assessed by measuring uptake of bromodeoxyuridine. The supernatant was analyzed for fibrogenic activating factor (FAF). Chromium- chloride was not cytotoxic to any of the cells. Chromium-trioxide, barium-chromate, lead-chromate, and potassium-dichromate caused dose dependent cytotoxicity in all cell types after 12 hours. Potassium- dichromate was the most toxic. Alveolar macrophages were the most sensitive, severe toxic injury being seen after 18 hours incubation with 0.67microM potassium-dichromate. Significant accumulations of chromium were seen in V79 and LEC cells treated with chromium-chloride and potassium-dichromate. Potassium-dichromate induced blebbing, membrane damage, and loss of filopodia, vesicles, and ruffles in alveolar macrophages. Supernatant from potassium-dichromate treated macrophages inhibited fibroblast growth and mitosis. The supernatant contained no FAF. Dithiothreitol, butylated-hydroxy- toluene, desferoxamine, and Trolox countered the effects of potassium-dichromate when present in the incubation medium. The authors conclude that Cr+3 and Cr+6 are taken up by V79 and LEC cells. Only Cr+6 compounds are cytotoxic to the examined cell systems. Cr+6 cytotoxicity may involve decreases in the intracellular glutathione pool resulting from inhibition of glutathione-reductase and liberation of oxygen radicals.

48
Noble C, Sina JF. USEFULNESS OF THE IN VITRO BONE MARROW COLONY-FORMING ASSAY IN CELLULAR TOXICOLOGY. In Vitro Toxicology 1993; 6(3):187-195.

The in vitro bone marrow colony-forming unit assay (CFU) was examined to determine its applicability and flexibility in addressing problems in toxicology. The defining features of an appropriate in vitro toxicologic method, ie., species differentiation, target-cell specificity, dose-response sensitivity and flexibility in compound exposure and timing, can, for at least one hemopoietic lineage (granulocyte-monocyte), be largely demonstrated in this assay. Test

conditions for two species, rat and dog, were studied and the assay tested using a number of compounds representing a variety of classes and actions. As with most in vitro assays, the in vitro bone marrow CFU assay is most applicable when comparing potential toxicities of related compounds with similar pharmaco-kinetics. Further refinement and complete validation could expand this methodology to include other species and hemopoietic lineages.

49
Segner H, Lenz D. CYTOTOXICITY ASSAYS WITH THE RAINBOW TROUT R1 CELL LINE. Toxicology In Vitro 1993; 7(4): 537-540.

For evaluation of the toxicity of aquatic pollutants, cultured fish cells are a potential alternative to fish bioassays. In this study, cells of the fibroblast-like R1 cell line from rainbow trout (Oncorhynchus mykiss) were exposed to a total of 30 organic and inorganic chemicals, in order to reveal possible correlations between R1 cytotoxicity and (a) toxicity to fish in vivo, (b) cytotoxicity data from other established fish cell lines, and (c) physicochemical parameters of the test agents. Cytotoxicity was assessed using the crystal violet (CV) staining technique, which determines the number of cells that are able to attach to the culture substratum during a 24-hr exposure period. For a broad spectrum of test compounds, including heavy metals as well as organic chemicals, there was good correlation of in vivo lethality and in

vitro cytotoxicity (n = 2 1, r = 0.84). However, of 21 chemicals, six (HgCl2, CuSO4, 2,4-dichlorophenol, 4-chloroaniline, chlorobenzene and phenol), were toxic to fish at concentrations at least 10 times lower than those in the cytotoxicity assay, whereas the in vitro assay was found to be more sensitive than the in vivo test for one chemical only (trichloroacetic acid). R1 midpoint toxicity values (CV50), on the other hand, closely agreed with cytotoxicity data reported for other fish cell lines (FHM, BF-2, GFS), both in absolute sensitivity and in relative ranking. For the 18 organic chemicals tested, a correlation between cytotoxicity in R1 cells and their octanol/water partition coefficient could be established. In conclusion, the R1 cell line seems to be a useful tool for screening studies in order to rank the toxicity of environmental pollutants or to evaluate structure-activity relationships.

The use of in vitro cytotoxicity assays as potential alternatives in assessing ocular irritation of surfactant mixtures was evaluated in a primary culture system of rabbit corneal epithelial cells. Two groups of surfactant mixtures, each with the same surfactant components in varying proportions, were studied. Cytotoxicity was determined by lactate dehydrogenase (LDH) enzyme leakage and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) dye reduction in the cell culture system. There was a good correlation between the cytotoxicity in vitro and the reported Draize eye irritation data within each group of the surfactant mixtures studied.

60
Nagami K, Maki E. IN VITRO CYTOTOXICITY TEST FOR ESTIMATING NON-OCULAR IRRITATION DOSE OF OPHTHALMIC SOLUTIONS. Cell Biol Toxicol 1993;9(2):107-18.

The in vitro cytotoxicity test for estimating the non-ocular irritation dose of ophthalmic solutions was investigated. In the in vitro test, normal human epidermal keratinocytes (NHEK) in a confluent monolayer were incubated for 48 hr in a medium with test compounds. The concentration of a test compound which causes a 50% reduction in NHEK viability was determined as IC50 by MTT colorimetric assay. For comparison, the in vivo rabbit ocular irritation tests were carried out by the standard Draize method. The maximum concentration, which did not show any ocular irritation, was determined as DS0. The results showed the correlation coefficient between the IC50 values and the DS0 values for 19 test compounds to be 0.82. However, the correlation coefficients for 10 compounds, which have IC50 values of less than 300 micrograms/ml, and for 7 alcohols were 0.99. The IC50-DS0 correlation curves obtained could be utilized as the critical concentrations for ocular irritation. These results suggest that our in vitro/in vivo test can estimate non-ocular irritation dose of the ophthalmic preparations in advance of the in vivo tests.

61
Babich H, Stern A, Munday R. IN VITRO CYTOTOXICITY OF 1,4-NAPHTHOQUINONE DERIVATIVES TO REPLICATING CELLS. Toxicol Lett (AMST)1993;69(1):69-75.

The acute cytotoxicities of a series of alkyl-1,4-naphtho-quinones (NQ) and of

irritation, because in vivo permeability coeffs. correlated with assocd. skin irritation.

74
Jacobs RR, Phanprasit W. AN IN VITRO COMPARISON OF THE PERMEATION OF CHEMICALS IN VAPOR AND LIQUID PHASE THROUGH PIG SKIN. Am Ind Hyg Assoc J 1993; 54(10):569-75.

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

75
Kanamori S, Tachihara R, Imai T, Aoki M, Sagara M, Nakayama K, Nakamura S. FUNDAMENTAL ANALYSIS OF IN VITRO CHEMOSENSITIVITY ASSAYS DEFINED BY THE INCORPORATION OF RADIOACTIVE MATERIALS. I. DETERMINATION OF SUITABLE EXPERIMENTAL CONDITIONS. Nippon Hifuka Gakkai Zasshi 1993;103(10):1273-8.

Fundamental anal. of in vitro chemosensitivity assays defined by the incorporation of radioactive materials was carried out for the purpose of determining their clin. application to skin cancer, especially malignant melanoma. Three human melanoma cell lines, G361, HMV-1, and Mewo, were used. Twenty-four h before harvesting, the cells were pulsed with 3H-thymidine for the measurement of DNA synthesis, 3H-uridine for RNA synthesis, or 3H-leucine for protein synthesis. All syntheses reached their highest levels on the 4th day in 1 .times. 104 cells in a 96-well flat-bottomed microculture plate. No increases in DNA, RNA, or protein synthesis were obsd. when the cell no. was 1

79
Monteiro-Riviere NA. USE OF THE ISOLATED PERFUSED SKIN MODEL IN DERMATOTOXICOLOGY. In Vitro Toxicology. J Molec Cell Toxicol 1993;5(4):219-233. (19 REFS)

Use of the isolated perfused porcine skin flap (IPPSF) model in dermatotoxicology was considered. Numerous studies which used the IPPSF were reviewed. Advantages of the IPPSF preparation included use of an isolated system with control over physiological parameters and perfusate composition, use of an anatomically intact and functional microcirculation responsive to topically applied compounds, use of a viable epidermis to allow assessment of cutaneous biotransformation, use of a large surface area of skin allowing human prototype transdermal products to be studied directly, use of a system amenable to detailed pharmacokinetic analyses, structural and functional similarities of the model skin preparation to human skin, provision of a humane alternative animal model system, and predictable extrapolations of the data to in-vivo percutaneous absorption estimates. The author concludes that the IPPSF appears to be a useful in-vitro model system for the simultaneous assessment of percutaneous absorption, penetration, and cutaneous toxicity. Future studies will focus on the relationships between early changes in inflammatory mediators and cytokine release and subsequent toxicologic changes.

80
Lewis RW, McCall JC, Botham PA, Kimber I. INVESTIGATION OF TNF-ALPHA RELEASE AS A MEASURE OF SKIN IRRITANCY. Toxicol In Vitro 1993;7(4):393-395.

Contact dermatitis is by far the most frequently reported occupational disease, with irritant dermatitis accounting for up to 80% of all cases. A wide variety of materials are capable of causing skin inflammation including soaps, cosmetics, pesticides, organic dyes, solvents and industrial chemicals and wastes. Skin irritation results from a complex series of events involving the development of an inflammatory response at the site of exposure. Cytokines are a family of proteins and glycoproteins that regulate immune and inflammatory responses; many are produced by epidermal cells. The present study examines the response of mouse epidermal strips to the cutaneous irritant sodium dodecyl sulphate (SDS). A time-dependent relationship was established for the release of the cytokine tumour necrosis factor-alpha, from epidermal keratinocytes

after treatment with 20% SDS. The potential value of this methodology for the detection of cutaneous irritants has been established. The utility of the approach for the identification in vitro of other materials of known in vivo irritant potential will be investigated.

81
Auton TR, Westhead DR, Woollen BH, Scott RC, Wilks MF. A PHYSIOLOGICALLY BASED MATHEMATICAL MODEL OF DERMAL

ABSORPTION IN MAN. Human & Experimental Toxicology 1994;13(1):51-60.

A sound understanding of the mechanisms determining percutaneous absorption is necessary for toxicological risk assessment of chemicals contacting the skin. As part of a programme investigat-ing these mechanisms we have developed a physiologically based mathematical model. The structure of the model parallels the multi-layer structure of the skin, with separate surface, stratum corneum and viable tissue layers. It simulates the effects of partitioning and diffusive transport between the sub-layers, and metabolism in the viable epidermis. In addition the model describes removal processes on the surface of the skin, including the effects of washing and desquamation, and rubbing off onto clothing. This model is applied to data on the penetration of the herbicide fluazifop-butyl through human skin in and in vitro. Part of this dataset is used to estimate unknown model parameter values and the remainder is used to provide a partial validation of the model. Only a small fraction of the applied dose was absorbed through the skin; most of it was removed by washing or onto clothing. The model provides a quantitative description of these loss processes on the skin surface.

82
Moir D, Marwood TM, Moody RP. IN VITRO CUTANEOUS METABOLISM OF DDT IN HUMAN AND ANIMAL SKINS. Bulletin of Environmental Contamination and Toxicology 1994;52(3):474-478.

No abstract.

83
Gordon VC, Harvell J, Bason M, Maibach H. IN VITRO

METHODS TO PREDICT DERMAL TOXICITY. Gad, S. C. (ED.). In Vitro Toxicology. IX+290P. Raven Press: New YORK, New York, USA. ISBN 0-88167-974-7. 1994;0(0):47-55.

No abstract.

84
Frantz SW, Beskitt JL, Tallant MJ, Futrell JW, Ballantyne B. GLUTARALDEHYDE: SPECIES COMPARISONS OF IN VITRO SKIN PENETRATION. J Toxic Cutan Ocul Toxic 1993;12(4):349-361.

It has been reported that the major portion of the applied dose was recovered from skin at the application site in previously conducted in vivo rat and rabbit pharmacokinetic studies with 14C-labeled glutaraldehyde. To investigate this finding further, and to compare penetration of glutaraldehyde through human skin with absorption data for animal skin preparations, the potential for in vitro skin penetration of (1,5-14C)glutaraldehyde (CAS #111-30-8) was evaluated with samples of excised skin from Fischer 344 rats, CD-1 mice, Hartley guinea pigs, New Zealand White rabbits, and humans (women undergoing reconstructive mammoplasty). A flow-through skin penetration chamber design was used and the aqueous glutaraldehyde concentrations of 0.75% and 7.5% used in the previous in vivo rat and rabbit percutaneous study were applied. The in vitro results indicated that glutaraldehyde did not penetrate human or animal skin to any substantial degree following application of either a 0.75% or a 7.5% aqueous solution. Averages of less than 0.5% of the applied radioactivity for the 0.75% solution and 0.7% of the applied dose for the 7.5% solution were recovered in the effluents for all animal species (range of 0.05 (female rats) to 1.73 (male mouse) for the 0.75% solution and 0.08 (male rat) to 1.55 (female rabbits) for the 7.5% solution). For human female skin, the recovery was approximately 0.2% in effluents for both applied glutaraldehyde concentrations. Under these in vitro experimental

conditions, glutaraldehyde did not penetrate human breast skin to any substantial degree, and this was largely due to a substantial portion of the dose binding to the skin during uptake. Thus, these results are consistent with previous reports and suggest that only a minimal amount of glutaraldehyde may be available for systemic uptake and distribution following cutaneous exposure. Based on these findings,

it was concluded that the potential for absorption may be less for humans than for common laboratory test species.

85
Eun HC. IN VITRO SKIN IRRITANCY APPLICATION OF KERATINOCYTES CELL CULTURE AND ITS CORRELATION WITH HUMAN PATCH TEST RESPONSES. Chonnam J Medical Sciences 1993;6(1):1-6.

No abstract.

86
Chang S-K, Dauterman WC, Riviere JE. PERCUTANEOUS ABSORPTION OF PARATHION AND ITS METABOLITES, PARAOXON AND P-NITROPHENOL, ADMINISTERED ALONE OR IN COMBINATION: IN VITRO FLOW-THROUGH DIFFUSION CELL STUDIES. Pesticide Biochemistry and Physiology 1994;48(1):56-62.

The percutaneous absorption of individual pesticides has been well studied. However, the absorption of a mixture of pesticides and decomposition products or metabolites has not received attention. The percutaneous absorption of parathion (PA) relative to its two metabolites, paraoxon (PO) and p-nitrophenol (PNP), was studied in vitro using weanling pig skin in a flow-through diffusion cell system. Concentrations of 4, 40, or 400 mug/cm2 of PA, PO, PNP, PA + PO (1:1), PA + PNP (1:1), and PO + PNP (1:1) in ethanol were applied topically. Environmental conditions such as air and perfusate temperature (37~C), relative humidity (60%), and flow rate (4 ml/hr) were controlled, and Kreb's-Ringer bicarbonate buffer with 4.5% bovine serum albumin media was used. The total absorption of PA, PO, and the mixed compounds increased as the dose increased, whereas the absorption efficiency (percentage of applied dose absorbed) decreased as the doses increased. For the most water-soluble metabolite, PNP, both total absorption and absorption efficiency increased as the dose increased. Except for the 4 mug/cm2 dose, the individual compounds followed the absorption order of PNP > PO > PA. For the mixed compounds, the rate of absorption followed the order PO + PNP > PA + PNP > PA + PO for all three dose combinations. However, after HPLC analysis of the perfusate, the amount of absorption of each component (PA, PO, and PNP) in mixed compounds was significantly different ($p < 0.05$). PNP dramatically enhanced PA

respectively. These values were significantly (P<0.01) higher than the rates of embryos cultured in Ham's F-10; 61.8%, 5.5% and 3.6% respectively. The deletion of CuSO4, ZnSO4, FeSO4 and hypoxanthine from Ham's F-10 significantly increased the rates of embryos reaching the 4-cell, blastocyst and hatched blastocyst stages to the extent comparable to those in alpha-MEM. In contrast, the addition of all or one of CuSO4, ZnSO4, FeSO4 and/or hypoxanthine to alpha-MEM significantly decreased the in vitro embryonic development. The strongest inhibition was observed when all of them were added. The developmental ability in alpha-MEM to which all of them were added was as low as that in Ham's F-10. These results suggest that the low developmental ability in Ham's F-10 may be mainly due to the deleterious effect of heavy metal ions and hypoxanthine. The toxic effect of heavy metal ions and hypoxanthine might be interpreted as the damage on embryos by an increased generation of oxygen radicals and the medium without constituents which may enhance the production of oxygen radicals seems to be desirable for the culture of mammalian embryos.

99
Schwetz BA. IN-VITRO APPROACHES IN DEVELOPMENTAL TOXICOLOGY. International Workshop on In Vitro Methods in Reproductive Toxicology, Ottawa, Ontario, Canada, May 19-20, 1992. Reprod Toxicol 1993;7(SUPPL 1): 125-127.

No abstract.

100
Schwetz BA, Harris MW. DEVELOPMENTAL TOXICOLOGY: STATUS OF THE FIELD AND CONTRIBUTION OF THE NATIONAL TOXICOLOGY PROGRAM. Environ Health Perspect 1993 Apr;100:269-82.

The NTP has conducted developmental toxicity studies on more than 50 chemicals, often in multiple species. Several chemicals caused developmental toxicity in the absence of any toxicity to the mother. Although hazard to humans is determined by the level of exposure to the chemical and its inherent toxicity, those agents that selectively disturb the development of the conceptus are of particular concern because other manifestations of toxicity would not warn the mother of overexposure. Whether the LOAEL (lowest-observed adverse effect

level) for maternal toxicity was high or low did not correlate with the potential of chemicals to cause developmental toxicity. The form of developmental toxicity that determined the LOAEL most frequently was decreased body weight in mice and rats, but not rabbits, where the LOAEL was determined more often by an increase in resorptions. Several in vitro and short-term tests appear promising as screens to predict the outcome of developmental toxicity studies in mammals. However, the only screens that have undergone formal validation studies are those evaluated by the NTP. Improvements in our ability to predict risk to humans have been limited by our knowledge of the mechanisms by which agents cause developmental toxicity. Thus, future growth is dependent on a better understanding of the biological processes that regulate normal development, therein providing the necessary framework for understanding mechanisms of abnormal development.

101
Fort DJ, Stover EL, Rayburn JR, Hull M, Bantle JA. EVALUATION OF THE DEVELOPMENTAL TOXICITY OF TRICHLOROETHYLENE AND DETOXIFICATION METABOLITES USING XENOPUS. Teratogenesis Carcinog Mutagen 1993; 13(1):35-45.

Potential mechanisms of trichloroethylene-induced developmental toxicity were evaluated using FETAX (Frog Embryo Teratogenesis Assay--Xenopus). Early Xenopus laevis embryos were exposed to trichloroethylene for 96 h in two separate definitive concentration-response assays with and without an exogenous metabolic activation system (MAS) and inhibited MAS. The MAS was treated with either carbon monoxide or cyclohexene oxide to modulate mixed- function oxidase (MFO) or epoxide hydrolase activity, respectively. Trichloroethylene metabolites: dichloroacetic acid, trichloroacetic acid, trichloroethanol, and oxalic acid were also evaluated in two separate definitive, static renewal tests. Addition of the MAS decreased the 96 h LC50 and EC50 (malformation) of trichloroethylene 1.8-fold and 3.8-fold, respectively. Addition of the carbon monoxide inhibited MAS decreased the developmental toxicity of activated trichloroethylene to levels approximating that of the parent compound. Cyclohexene oxide-inhibited MAS substantially increased the developmental toxicity of trichloroethylene. In addition, each of the metabolites tested were significantly less developmental toxic than the parent

compound, trichloroethylene. Results indicate that a highly embryotoxic epoxide intermediate, trichloroethylene oxide, formed as the results of MFO mediated metabolism may play a significant role in the developmental toxicity of trichloroethylene in vitro.

102
Combes RD, Willington SE, Zajac W, Toraason M, Bohrman JS, Krieg E, Langenbach R. EVALUATION OF THE V79 CELL METABOLIC CO-OPERATION ASSAY AS A SCREEN IN VITRO FOR DEVELOPMENTAL TOXICANTS. Toxicology In Vitro 1992;6(2):165-74.

Inhibition of intercellular communication is proposed to be one of several possible mechanisms of teratogenesis. 38 coded compounds were tested for their effect on intercellular communication in the V79 cell metabolic co-operation assay. Test chemicals were selected from a list of 47 agents recommended for the evaluation of assays in vitro for developmental toxicants. In addition to testing the effects of chemicals on intercellular communication, a separate cytotoxicity assay determined the concentration of each chemical that inhibited clonal expansion of V79 cells. Seven of the 29 designated teratogens were positive for inhibition of intercellular communication in the V79 assay. Additionally, four teratogens and one non-teratogen inhibited intercellular communication at only a single concentration or at cytotoxic concentrations and were scored as equivocal. Therefore, the sensitivity of the V79 assay for teratogens was 24% (seven of 29 teratogens tested positive), or 38% if the four equivocal chemicals are considered positive. None of the nine non-teratogens unequivocally inhibited intercellular communication, resulting in a specificity of 100%, which decreased to 89% when the single equivocal score was considered positive. The overall accuracy for correctly identifying teratogens and non-teratogens was 42% when equivocal chemicals were considered negative, and 50% if they were considered positive in the V79 assay. The results demonstrate that despite relatively low accuracy regarding a diverse group of developmental toxicants, chemicals that did inhibit intercellular communication under the present conditions had a high probability of being a teratogen. The low accuracy reported here contrasts with earlier reports on the assay and possible reasons for this are discussed.

during two stages of organogenesis to CB at final concentrations of 0 (control), 10, 25, 50, 100, and 200 micrograms/ml. Embryos were evaluated for heart rate (HR), malformations, and somite number, and embryos and visceral yolk sacs (VYSs) were assayed for total protein content as a measure of overall growth. Neurulating (3-6 somite) embryos were malformed and growth retarded by exposure to CB concentrations > or = 25 micrograms/ml, with decreased VYS growth at > or = 50 micrograms/ml and decreased HR at > or = 100 micrograms/ml CB. Early limb-bud stage (20-25 somite) embryos were malformed at CB concentrations > or = 50 micrograms/ml and growth retarded at > or = 100 micrograms/ml, with decreased VYS growth at 200 micrograms/ml and decreased HR at > or = 100 micrograms/ml CB. Thus, CB produces dysmorphogenesis in mouse embryos in vitro, and neurulating embryos are somewhat less sensitive than early limb-bud stage embryos. The concentrations of CB that interfere with normal embryonic development are within the range of human blood levels measured following multiple doses of CB. Preparations containing CB should be used with caution during pregnancy, particularly when repeated dosing may allow accumulation of CB to potentially embryotoxic levels.

112
Cumberland PF, Richold M, Parsons JF, Pratten MK. INTRAVITELLINE INJECTION OF CULTURED RAT EMBRYOS: AN IMPROVED METHOD FOR THE IDENTIFICATION OF CYTOTOXIC AND NON-CYTOTOXIC TERATOGENS. Toxicology in Vitro 1992; 6(6):503-8.

A preliminary study of a novel developmental toxicity screen has been carried out. The technique involves the direct injection into the vitelline circulation of the 11.5-day rat conceptus, by-passing the metabolically active visceral yolk sac. The evaluation was performed blind using four coded model compounds: sulphanilamide (non-cytotoxic, non-teratogen), retinoic acid (teratogen) and methotrexate and cyclophosphamide (both cytotoxic teratogens). Seven parameters of teratogenicity and cytotoxicity were measured (yolk sac diameter, crown-rump length, somite number, yolk sac protein, yolk sac DNA, embryo protein, embryo DNA) and morphological abnormalities were also noted. The results showed that this technique successfully identified the developmental toxins and, moreover, differentiated between teratogens and cytotoxic teratogens. Additionally, the results show that

117
Berces J, Otos M, Szirmai S, Crane-Uruena C, Koteles GJ. USING THE MICRONUCLEUS ASSAY TO DETECT GENOTOXIC EFFECTS OF METAL IONS. Environ Health Perspect 1993;101(Suppl 3):1-13.

The lymphocyte micronucleus assay was used to measure the av. frequency of micronuclei in a population and thus assess genotoxic effects. Data from 174 persons give an av. value of 16.4, and a slight age-dependence was obsd. To detect combined environmental mutagen injuries, the micronucleus assay was used to study the effects of metal compds. Cd ions increased the micronucleus frequency linearly after incubation with whole blood in vitro with 10-6-10-3 M concns. for 30 min. Similarly, a linear increase in micronucleus frequency was detected with 10-3-10-1 M Hg ions. Concerning the biol. effect of Se, it was found that NaSeO3 nor SeO2 induced increases at 10-7-10-6 M; 10-5 M caused a slight increase; 10-4 M, however, destroyed the cells. Thus, the human lymphocyte micronucleus test can be used to assess genotoxic injuries due to environmental effects in human lymphocytes.

118
Ellard S, Parry EM. A MODIFIED PROTOCOL FOR THE CYTOCHALASIN B IN VITRO MICRONUCLEUS ASSAY USING WHOLE HUMAN BLOOD OR SEPARATED LYMPHOCYTE CULTURES. Mutagenesis 1993;8(4):317-20.

A modified protocol is described for the in vitro anal. of micronuclei in whole blood or sepd. lymphocyte cultures. The induction of binucleate cells by various concns. of cytochalasin B (3, 4.5, or 6 mug/mL) was examd. at 2 harvest times (68 or 72 h). An optimal yield was obtained by adding cytochalasin B at a dose of 6 mug/mL to cultures 44 h after initiation with harvest 24 h (whole blood) or 28 h (sepd. lymphocytes) later. Cytocentrifuge prepns. of lymphocytes (sepd. from whole blood using com. prepns. of Ficoll either at the commencement of the assay or upon harvest) were stained with Acridine Orange. Using this method, cytokinesis-blocked lymphocytes remain intact and micronuclei are readily identified. The method is suitable for both whole blood and sepd. lymphocyte cultures, thus allowing direct comparisons of sensitivity to genotoxic agents.

122
Hoflack JC, Ferard JF, Vasseur P, Blaise C. AN ATTEMPT TO IMPROVE THE SOS CHROMOTEST RESPONSES. J Appl Toxicol 1993;13(5):315-319. (19 REFS)

A method for studying direct toxic effects in the SOS Chromotest was developed and tested. Alkaline-phosphatase and beta-galactosidase activities are measured in the SOS Chromotest; differential inhibition of the enzyme activities may wrongly indicate genotoxicity, due to direct toxic effects. Identification of the false positive response frequently observed with complex mixtures was possible using a simple additional manipulation. The SOS Chromotest bacteria were challenged with samples having previously shown a positive genotoxic response just before the enzyme activities were estimated colorimetrically. The procedure was applied to leachates of ten industrial waste samples, which initially gave positive genotoxic responses. The SOS Chromotest was compared with a miniaturized version using microplates. The two methods produced identical results in nine samples, eight of which were positive and 1 negative. Discrepancies between the results for the standard and the miniaturized procedures were eliminated by use of this method. A flowchart was presented for the interpretation of SOS Chromotest results.

123
Rank J, Jensen A-G, Skov B, Pedersen LH, Jensen K. GENOTOXICITY TESTING OF THE HERBICIDE ROUNDUP AND ITS ACTIVE INGREDIENT GLYPHOSATE ISOPROPYLAMINE USING THE MOUSE BONE MARROW MICRONUCLEUS TEST, SALMONELLA MUTAGENICITY TEST, AND ALLIUM ANAPHASE-TELOPHASE TEST. Mutat Res 1993;300(1):29-36. (15 REFS)

The genotoxic effects of the formulated commercial herbicide Roundup and its active agent glyphosate-isopropylamine-salt (GIS) were examined using the mouse bone marrow micronucleus test, the Salmonella mutagenicity test using strains (TA-98) and (TA-100), and the Allium anaphase/telophase test. NMRI-Bom-mice were used for the bone marrow micronucleus assay. The allium anaphase/telophase assay was performed using root cells from Allium-cepa. The findings indicated that Roundup, a mixture of several agents, can induce weak mutations in both strains of Salmonella-

typhimurium tested, and can induce chromosome aberrations in A-cepa meristem root cells at concentrations close to the level of toxicity. Roundup showed a weak mutagenic effect for concentrations of 360 micrograms/plate in (TA-98) without S9 and 720 micrograms/plate in (TA-100) with S9. The anaphase/telophase Allium test gave no evidence for any effect caused by GIS, but did show a significant increase in chromosome aberrations following treatment with Roundup at 1.44 and 2.88 milligrams/liter when calculated as glyphosate-isopropylamine. Disturbances of the spindle were the most frequent aberrations found.

124
Gollapudi BB, Linscombe VA, McClintock ML, Sinha AK, Stack CR. TOXICOLOGY OF DIETHYLENE GLYCOL BUTYL ETHER. GENOTOXICITY EVALUATION IN AN IN VITRO GENE MUTATION ASSAY AND AN IN VIVO CYTOGENETIC TEST. J Amer Coll Toxicol 1993;12(2):155-159. (7 REFS)

The genotoxicity of diethylene-glycol-butyl-ether (DGBE) was assessed using an in-vitro gene mutation assay and an in-vivo cytogenetic test. The in-vitro test was the Chinese-hamster-ovary (CHO) cell forward gene mutation at the hypoxanthine-guanine-phosphoribosyl-transferase (HGPRT)locus. Mutation frequencies were statistically evaluated by pairwise tests comparing each dose level to a negative control, and by linear and quadratic trend analysis. The in-vivo assay used the CD-1 mouse bone marrow micronucleus test for evaluation of cytogenetic damage. Polychromatic erythrocytes (PCE) were examined to determine the incidence of micronucleated (MN) PCE, and the relative proportions of PCE and normochromatic erythrocytes were estimated. Results showed that the DGBE was not toxic to CHO cells even at concentrations of 5,000 micrograms per milliliter (microg/ml). At 1,000microg/ml, mutation assays showed that in the absence of S-9, DGBE did not significantly increase the frequencies of 6-thioguanine resistant mutants (which represented the HGPRT phenotype. At 3,000microg/ml, DGBE induced a significantly elevated mutation rate, which however, was reduced to a nonsignificant level when compared with the historical negative value. In MN assays at 24, 48, and 72 hours post treatment, no significant effects on the incidence of MN-PCE were observed. No adverse effects on cell kinetics were recorded. The authors conclude that the micronucleus test and the CHO cell gene mutation assay do not indicate a genotoxic

potential for DGBE.

125
Albertini S, Brunner M, Wurgler FE. ANALYSIS OF THE SIX ADDITIONAL CHEMICALS FOR IN VITRO ASSAYS OF THE EUROPEAN ECONOMIC COMMUNITIES' EEC ANEUPLOIDY PROGRAMME USING SACCHAROMYCES CEREVISIAE D61.M AND THE IN VITRO PORCINE BRAIN TUBULIN ASSEMBLY ASSAY. Environmental and Molecular Mutagenesis 1993;21(2):180-192. (64 REFS)

The genotoxicities of acetaldehyde, benomyl, diethylstilbestrol(DES), diethylstilbestrol-dipropionate (DESdP), griseofulvin, and 2-mercaptoethanol(2ME)were studied in-vitro. The compounds were part of the chemicals to be included in the European Economic Communities database on aneuploidy. The compounds were tested for their ability to induce chromosome malsegregation and cycloheximide resistant colonies at the ilv1-92 locus in Saccharomyces- cerevisiae-D61.M. Concentrations ranged up to 333 micrograms per milliliter (microg/ml) for the solids and 1.75 microliters per milliliter (microl/ml) in the case of the liquids. Dimethyl- sulfoxide (DMSO) or ethanol was used as the solvent. Incubation times were 16 hours at 28 degrees-C or 4 hours at 4 degrees plus 16 hours at 28 degrees plus 4 hours at 4 degrees. DES, griseofulvin, and 2ME at 0 to 50 millimolar were tested for their ability to inhibit tubule assembly in the porcine brain tubulin assembly assay. Acetaldehyde caused dose dependent increases in chromosome malsegregation in S-cerevisiae. The lowest effective doses (LEDTs) measured in two experiments were 1.25 and 0.75microl/ml. A weak induction of cycloheximide resistant mutantcells was also detected. Benomyl dissolved in DMSO caused a dose dependent increase in S-cerevisiae malsegregation. The LEDT was 30microg/ml. Benomyl did not induce any cycloheximide mutant cells. DES was not genotoxic when tested inDMSO. When dissolved in ethanol, DES caused dose related increases in the chromosome malsegregation frequency. The LEDT was 13microg/ml. DESdP, griseofulvin, and 2ME were not genotoxic in S-cerevisiae. DES and griseofulvin inhibited the assembly process in the porcine brain tubulin assembly assay. The concentrations inhibiting tubulin assembly by 30% were 12.5 and 100 micromolar, respectively. 2ME was inactive.

126
Khan MA, Jostes RF, Cross FT, Rithidech K, Brooks AL.

MICRONUCLEUS A BIOMARKER OF GENOTOXIC DAMAGE INDUCED IN VIVO AND IN VITRO FROM RADIATION AND CHEMICAL EXPOSURE. Thirty-first Hanford Symposiom on Health and the Environment, Richland, Washington, USA, October 20-23, 1992. J Toxicol Environ Health 1993;40(2-3):455-456.

No abstract.

127
Crespi CL, Penman BW, Gonzalez FJ, Gelboin HV, Galvin M, Lagenbach R. GENETIC TOXICOLOGY USING HUMAN CELL LINES EXPRESSING HUMAN P-450. Biochemical Society 647th Meeting on Chromosomal Abnormalities in Cancer Cells: Identification of Molecules Improtant for Tumour Development, Sheffield, Engnald, UK, July 20-23, 1993. Biochemical Society Transactions 1993;21(4):1023-1028.

No abstract.

128
Adler I-D, Parry JM. DEVELOPMENT OF SCREENING TESTS FOR ANEUPLOIDY INDUCTION BY ENVIRONMENTAL POLLUTANTS. Environ Health Perpsec 1993;101(SUPPL 3):5-9.

No abstract.

129
Godet F, Vasseur P, Babut M. IN VITRO AND IN VIVO GENOTOXICITY TESTS FOR STUDYING CONTAMINATED AQUATIC ENVIRONMENTAL SAMPLES. Revue des Sciences de L'eau 1993; 6(3):285-314.

This review deals with in vitro and in vivo genotoxicity bioassays carried out to evaluate the genotoxic potential of polluted environmental samples: continental and marine waters, domestic and industrial wastewaters, aquatic sediments and sludges of urban or industrial wastewater treatment plants. The end-points of the in vitro and in vivo assays are: genetic alterations, i.e. reverse and forward mutations, DNA adducts or chromosomic damages, i.e. chromosomic aberrations (AC), micronuclei (MN) and sister chromatid exchanges (SCE). The in vitro assays generally detect adverse effects an DNA only after concentration or extraction of micropollutants. They constitute miniaturized tools, rapid and easy to use, thus well-suited for large screening studies. In vitro genotoxicity bioassays requiring only small volumes of

samples are therefore systems of choice for testing concentrates or extracts from environmental contaminated samples. Among the in vitro assays reviewed, the Salmonella typhimurium gene mutation test is the most often used to assess the genotoxic potential of contaminated samples. However, genotoxicity tests performed on eukaryotic cell cultures are more relevant than those using bacteria for evaluating environmental pollution. The use of fish cell lines appears superior to the use of mammalian cells for assessing an aquatic impact. In vitro bioassays, whether performed on prokaryotic or eukaryotic cells, are limited for predicting the possible impact of genotoxic pollutants on the environment. It is clear that it is difficult to extrapolate in vitro bioassay results to higher organisms in which the response obtained integrates effects of complex metabolizing systems, hormonal regulation and immunological defenses. Therefore, genotoxicity studies performed with aquatic organisms such as molluscs (Mytilus sp.), fish (Umbra pygmaea, Notobranchius rachowi) or amphibians (Pleurodeles walti) appear more representative of environmental conditions. The genotoxicity end-points of in vivo assays are mainly cytogenetic damage such as the SCE, AC or MN but also take into account DNA adducts. Direct testing of environmental samples without preconcentration is possible with in vivo assays. This means that factors such as bioavailability and metabolism will be integrated directly in the response of these assays. Hence, these in vivo assays are more sensitive than in vitro genotoxicity tests. However, in vivo tests require important volumes of sample and it will be difficult or almost impossible to apply them for testing concentrates or sample extracts, generally only available in small quantities. An interesting area of application of in vivo assays is field studies and ecoepidemiology. In this respect, they would constitute an a posteriori control system of pollution effects, assuming that suitable control areas are available to eliminate the influence of confounding factors. As a general conclusion, it is important to emphasize the interest of using both in vitro and in vivo bioassays for evaluating the genotoxicity of contaminated environmental samples. This rationale is based on the fact that in vitro bioassays are well adapted for genotoxicity screening or concentrates and extracts testing, while in vivo tests are interesting because of their better representativity in terms of environmental conditions of exposure to pollutants.

130
Geard CR. CYTOGENETIC ASSAYS FOR GENOTOXIC AGENTS. Lens Eye Toxic Res 1992; 9(3-4):413-28.

The induction of genetic damage has clear and dramatic implications for human health, with teratogenic, mutagenic, cataractogenic and carcinogenic consequences resulting from cellular chromosomal alterations in appropriate tissues. When analysing the potential of an agent to initiate genetic damage or in evaluating possible incumbent genomic damage a variety of complementary assays may be employed. These apply to cells in vitro, to in vivo assessments involving small mammals and most importantly to derived human cells and tissues including those of ocular origin. Cytogenetic assays have the important advantage that they enumerate damage at the level of the individual cell. Assays involving the examination of chromosomal aberrations at mitosis, of cells prior to mitosis using the technique of premature chromosome condensation, of micronuclei in post-mitotic cells and of sister chromatid exchanges will be described. The development of human chromosome specific probes and fluorescent in situ hybridisation (FISH) techniques combine the resolution of molecular biology with classical cytogenetics in a powerful approach to defining genomic change and its consequences. These techniques and assays can be further augmented by in situ cytometry such that overall a number of parameters can be quantified involving cellular kinetics, clastogen and/or aneugen definition and ultimately the establishment of dose response relationships. A rational basis for avoidance or control, for intervention or for defining probable cause of the role of genotoxicants in the development of human disease can then be established.

131
Czeczot H, Kusztelak J. A STUDY OF THE GENOTOXIC POTENTIAL OF FLAVONOIDS USING SHORT-TERM BACTERIAL ASSAYS. Acta Biochim Pol 1993;40(4):549-54.

Genotoxic activities of flavonoids (quercetin, rhamnetin, isorhamnetin, apigenin, luteolin) were investigated using two short-term bacterial assays. In the "repair test" in Salmonella typhimurium (strains TA1538 uvrB- and TA1978 uvrB+) the flavonoids studied did not introduce any damage into the DNA recognized by UvrABC nuclease (correndonuclease II). The results of the SOS-Chromotest in Escherichia coli K-12 strains

PQ37 (tag+, alk+) and PQ243 (tagA, alkA) indicated that flavonoids only weakly induced the SOS system. The addition of a liver activation system (S9 mix) did not increase the mutagenic effect of the flavonoids tested. Two compounds: rhamnetin, isorhamnetin and their putative metabolites formed in the presence of the S9 mix did not alkylate DNA at N-3 of adenine.

132
Zeiger E. Series: 'CURRENT ISSUES IN MUTAGENESIS AND CARCINOGENESIS.' NO. 42. STRATEGIES AND PHILOSOPHIES OF GENOTOXICITY TESTING: WHAT IS THE QUESTION? Mutat Res 1994;304(2):309-14. (REFS: 23)

A number of statements concerning the uses and effectiveness of in vitro and in vivo genetic toxicity tests have recently been made. Certain of these statements are examined using genetic toxicity and carcinogenicity data available in the literature.

133
Chetelat A, Albertini S, Dresp JH, Strobel R, Gocke E. PHOTOMUTAGENESIS TEST DEVELOPMENT: I. 8-METHOXYPSORALEN, CHLORPROMAZINE AND SUNSCREEN COMPOUNDS IN BACTERIAL AND YEAST ASSAYS. Mutat Res 1993;292(3):241-50.

Two in vitro genotoxicity tests have been adapted to the evaluation of photomutagenic activity of test compounds. The study was initiated to obtain an experimental basis relating to newly proposed guidelines of the EC which request the screening of UV-absorbing compounds, for example, those employed in sunscreen preparations, for their photomutagenic potential. The well established photomutagens 8-methoxypsoralen and chlorpromazine were used to define relevant test protocols. The compounds were evaluated with the Ames test and the Saccharomyces cerevisiae D7 test for gene conversion. The influence of various parameters such as UV light sources, spectral composition, UV sensitivity of the test systems, absorbance by test materials and different exposure conditions is indicated. Two exemplary screening experiments with cosmetic ingredients are presented. Both test systems can be employed for the evaluation of compounds for photomutagenic activity although the standard excision-deficient strains of S. typhimurium pose problems because of their high UV sensitivity. The present experience in this complex

response was strongly inhibited by monoclonal antibodies to major histocompatibility complex (MHC) class I and II, CD4 antigens and ICAM-1 and LFA-3 adhesion molecules. Furthermore, we found that fresh LC can prime T cells to TNP, as revealed by a significant secondary T-cell proliferation after restimulation of the recovered T lymphocytes by fresh hapten-modified autologous LC. Nevertheless, the ability of these fresh LC to stimulate in vitro secondary hapten-specific T-cell proliferation was very limited in comparison with that of 2-day incubated Langerhans' cells. After secondary stimulation with TNP-cultured LC, sensitized T cells could be non-specifically expanded without losing hapten specificity. The TNP-specific T-cell lines were mostly of the CD4+ phenotype. The present findings extend previous studies in the mouse, showing that cultured LC are potent antigen-presenting cells (APC) in primary hapten-dependent proliferation assays. Furthermore, this in vitro priming assay, using cultured human Langerhans' cells as APC, might be useful to analyse the early steps of T-cell sensitization and subsequently to develop in vitro predictive tests allowing detection of sensitizing compounds.

138
Pape W JW, Degwert J, Steckel F, Hoppe U.
IMMUNOCOMPETENT CELLS FOR IN VITRO SCREENING OF SKIN IRRITATION. Toxicology In Vitro 1993;7(4):389-392.

The present studies were aimed at evaluating procedures for assessing the immunomodulatory effects of chemicals and preparations on macrophage differentiation and lymphocyte proliferation in cell cultures. The effects of 10 drugs and anti-inflammatory agents were monitored by determining thymidine incorporation into phytohaemagglutinin (PHA)-stimulated T cells in the lymphocyte transformation test (LTT) and the expression of two surface antigens on macrophages in the macrophage differentiation assay (MDA). One antigen was found on macrophages in acute inflamed tissue. The other was detected on those found in recovering tissue. These parameters were compared with mean skin irritation scores for 12 known cosmetic products from epicutaneous patch testing. Finally, these parameters were also used to study six cosmetic test formulae with unknown irritation potentials subjected to blind testing during phase 2 of the "CTFA Evaluation of Alternatives Program". Immunosuppressive agents were detected in both systems. Agents, thought

very sensitive to immunomodulation by lead, whereas human cells were relatively resistant. It is suggested that direct interspecies comparisons of immunological effects due to chemical treatment in vitro can provide a greater understanding of the relationship between animal and human data, which will improve the confidence of extrapolation from findings in laboratory animals to human health risk.

141
Fautz R, Miltonburger HG. IMMUNOTOXICITY SCREENING IN VITRO USING AND ECONOMICAL MULTIPLE ENDPOINT APPROACH. Toxicol In Vitro 1993;7(4):305-310.

An economical multiple endpoint in vitro test battery has been developed for screening chemically induced immune dysfunction. Bearing in mind the complexity of the immune system, different types of immunocompetent cells were used. Cofactor-fortified liver homogenate obtained from rats pretreated with Aroclor (S-9 mix) was employed as an in vitro metabolizing system. The following principal screening design was applied. Immuno-competent cells (peritonea cells and splenocytes) obtained from female C57B1 mice were treated in vitro for 1 hr. For metabolic activation, chemicals were pretreated with S-9 mix for 2 hr. After the incubation period the cells were washed and different immune function assays (antibody-dependent phagocytosis and lipopolysaccharide-induced release of turnout necrosis factor of thioglycollate-elicited peritonea) macrophages; natural killer cell activity, T- and B-cell blastogenesis, and B-cell antibody synthesis of spleen cell suspensions) were performed. For economy the different spleen cell functions were tested in parallel with aliquots of cells derived from the same chemically treated culture. As an additional parameter the survival of the cells was determined routinely after treatment during all assays. Different chemicals (e.g. tributyltinoxide, 7,12-dimethylbenzanthracene, lead acetate, cyclophosphamide, dexamethasone) were assessed using this system. The results indicate that the in vitro test battery described is a suitable tool for immunotoxicity screening.

METABOLISM/XENOBIOTICS
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Zhang XX, Chakrabarti S, Malick AM, Richer CL. EFFECTS OF DIFFERENT STYRENE METABOLITES ON

CYTOTOXICITY, SISTER CHROMATID EXCHANGES AND CELL CYCLE KINETICS IN HUMAN WHOLE BLOOD LYMPHOCYTES IN VITRO. Mutat Res 1993; 302(4):213-18.

Five metabolites of styrene were tested in vitro for their cytotoxic effects, induction of SCEs and changes in cell-cycle progression in cultured human blood lymphocytes. Fresh heparinized peripheral blood (0.3 mL) from normal volunteers was cultured for a total of 72 h in 5 mL of RPMI 1640 medium contg. 10% fetal calf serum, 0.1% garamycine, 1% glutamine and 1% phytohemagglutinin. Styrene-7,8-oxide (SO), styrene glycol (SG), phenylglyoxylic acid (PGA), S-(1,2-dihydroxyethyl)glutathione (PEG) (a glutathione conjugate of styrene oxide), N-acetyl-S-(1,2-phenyl-2-hydroxyethyl)cysteine (NAPEC) in DMSO were injected into the cultures 36 h after initial culture, so that the exposure time for test metabolites was 36 h. The final concentration of SO was 100 muM and those of the other metabolites were 500 mum. Twenty-four h before harvest, BrdU (10 mug/mL) was added into the cultures for assessing cytogenetic endpoints. SO showed significant induction of SCEs and cell-cycle delay as well as a significant decline of cell survival. The same phenomena, but of less magnitude, were also observed with NAPEC, a cysteine deriv. of SO. On the other hand, SG, PGA and PEG failed to produce any significant changes of these endpoints compared to the control. Thus, the present results have demonstrated that, in addition to SO, NAPEC possess some cytogenotoxic potential and hence, these 2 metabolites together could contribute to the genotoxicity of styrene in human blood lymphocytes.

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Rogiers V, Vercruysse A. RAT HEPATOCYTE CULTURES AND CO-CULTURES IN BIOTRANSFORMATION STUDIES OF XENOBIOTICS. Toxicol 1993;82(1-3):193-208.

Long-term culture of hepatocytes could represent a suitable in vitro model for biotransformation studies of xenobiotics. At present however, no ideal culture system can be proposed since, in all existing models, phenotypic changes occur, affecting selectively some components of phase I and/or phase II xenobiotic metabolism. From the authors' own results and recent studies of several other investigators, carried out on rat hepatocytes, it becomes clear that four groups of factors may affect biotransformation capacity: soluble medium factors, extracellular matrix components,

micronucleus assay are necessary to clarify its role in mutagenicity testing.

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Blakey DH, Maus KL, Bell R, Bayley J, Douglas GR, Nestmann ER. MUTAGENIC ACTIVITY OF 3 INDUSTRIAL CHEMICALS IN A BATTERY OF IN VITRO AND IN VIVO TESTS. Mutat Res 1994; 320(4):273-83.

Three chems. were selected for mutagenicity testing from a priority list, based on prodn. vol. and available mutagenicity data. Propargyl alc. (PA), 2-nitroaniline (NA), and 5-methyl-1H-benzotriazole (MBT) were selected for testing using the approach recommended in the Health Protection Branch Genotoxicity Guidelines. The battery of tests included the Salmonella/mammalian microsome mutation assay, the in vitro chromosomal aberration assay, and the bone-marrow micronucleus assay. The results indicate that 2 of the 3 chemicals, PA and NA, were clastogenic in vitro. Both PA and NA induced chromosomal aberrations in CHO cells in vitro with and without metabolic activation, while none induced reverse mutations detectable with the Salmonella/mammalian microsome assay. Because PA and NA were found to be in vitro clastogens, they also were tested in the mouse bone marrow micronucleus assay. NA induced a small increase in micronuclei in males but not females. PA did not induce an increase in micronuclei.

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Bean CL, Galloway SM. EVALUATION OF THE NEED FOR A LATE HARVEST TIME IN THE ASSAY FOR CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY CELLS. Mutat Res 1993; 292(1):3-16.

The effect of harvest time on the results of in-vitro assays using Chinese-hamster ovary (CHO) cells was studied. CHO cells were maintained in culture and exposed to various chemicals for 3 hours and harvested 20 or 44 hours after the start of the exposure. Cells were then assessed for toxicity, number of cells in metaphase, and number of chromosomal aberrations. Benzo(a)pyrene (BP), cadmium-sulfate, chlorambucil (CAB), 2,6-diaminotoluene (2,6-DAT), 4-nitroquinoline (4-NQO), and mitomycin-C (MMC) induced considerable amounts of aberrations at 20 hours but few at 44 hours while 2-aminobiphenyl (2-ABP), eugenol (EUG), and 8-hydroquinoline (8-HQ) induced similar numbers of