1994 No. 4
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.

CNS
1
de Boer AG, de Vries HE, de Lange EC M, Danhof M, Kuiper J, Breimer DD. DRUG TRANSPORT TO THE BRAIN: IN VITRO VERSUS IN VIVO APPROACHES. J Controlled Release 1994;28(1-3):259-63.

This paper summarizes the applicability of in vitro and in vivo methods to assess drug (e. g. peptide drug) transport to the brain. In vitro methods use culture of confluent monolayers of bovine brain endothelial cells which represent the blood-brain barrier (BBB) and confocal laser scanning microscopy. In vivo

methods use BBB-clearance model and microdialysis in rats. Ascertaining selectivity in BBB-transport in disease states is emphasized.

CARCINOGENICITY
2
Laduca JR, Sinha DK. IN VITRO CARCINOGENESIS OF MAMMARY PITHELIAL CELLS BY N-NITROSO-N-METHYLUREA USING A COLLAGEN GEL MATRIX CULTURE. In Vitro Cellular & Developmental Biology Animal 1993;29A(10):789-794.

Carcinogenesis is a lengthy process which eventually culminates in the transformed phenotype, cancer. However, much remains to be defined about the process of transformation. In vivo models for the study of the carcinogenic process present limitations because it is not possible to detect the premalignant stages in the animals. An in vitro model, on the other hand, facilitates the study of the carcinogenic process because it enables one to dissect out the crucial events required for carcinogenesisto occur. As carcinogenesis is believed to be a multistep process; initiation, promotion, and progression, a multistep, in vitro system has been devised in our laboratory to mimic each of these stages. We have previously shown the formation of "microtumors" in collagen gels, induced by 7,12-dimethylbenz(a)anthracene. In the present study the direct acting water soluble, mammary carcinogen, N-nitroso-N-methylurea (NMU) was used for tumorigenesis of mammary epithelial cells in culture. Mammary epithelial cells from virgin Sprague-Dawley rats were propagated and exposed to single or multiple doses of NMU while growing as a monolayer in glass petri dishes (initiation). Initiated cells were then plated into a collagen gel matrix culture. Prolonged growth in the collagen gels afforded for the progression of the transformed cells into discernable microtumors in the three-dimensional matrix of the collagen. The morphology of these "tumors" was determined by histologic sections of the gels. Fewer, if any, such structures existed in the untreated gels.

CARDIOTOXICITY
3
Parrish AR, Wishnies SM, Dorr RT, Gandolfi AJ, Brendel K.
COCULTURE OF RABBIL LIVER AND MYOCARDIAL SLICES: POTENTIATION OF THE CARDIOTOXICITY OF ALLYL ALCOHOL BUT NOT ALLYLAMINE. In Vitro Toxicology 1994;7(1):53-57.

The coculture of precision-cut rabbit liver and myocardial slices as a tool to investigate metabolite-mediated cardiotoxicity was examined by determining the effect of metabolic activation on the cardiotoxicity of allylamine (AAM) and allyl alcohol (AOH). AAM is activated by benzylamine oxidase, a predominantly cardiovascular enzyme, to acrolein. Acrolein is also produced from AOH by alcohol dehydrogenase, which is primarily found in the liver. Under suitable conditions, the coculture of liver and myocardial slices had no detrimental effect on the viability of either tissue type, as assessed by protein synthesis. AAM and AOH (10-7 and 10-5 M) were not significantly toxic to liver slices. AAM, but not AOH, was toxic to myocardial slices in organ culture. In coculture, 10-5 M AOH was significantly toxic in comparison to both control myocardial slices and myocardial slices exposed to the same concentration of AOH without the presence of liver slices. These results indicate that the coculture of liver and myocardial slices is a viable approach to the in vitro study of metabolism-mediated toxicity in the heart.

CELL CULTURE
4
Bolon B, Dorman DC, Bonnefoi MS, Randall HW, Morgan KT.
HISTOPATHOLOGIC APPROACHES TO CHEMICAL TOXICITY USING PRIMARY CULTURES OF DISSOCIATED NEURAL CELLS GROWN IN CHAMBER SLIDES. Toxicol Pathol 1993;21(5):465-79.

In the present work, "tissue" and cell morphol. of control and toxicant-treated primary dissocd. cerebrocortical cell cultures from fetal mice were examd. using phase-contrast and bright-field microscopy. In untreated control cultures, a reproducible sequence of developmental events included cellular reaggregation, intercolony bridging with cell migration, and neuronal apoptosis, with maturation yielding confluent monolayers contg. both neurons and glia. Because even mature cultures had regions of varying differentiation, an understanding of the normal developmental sequence was essential when assessing toxicant-treated cultures for damage. Chems. induced neuronotoxic, gliotoxic, and cytotoxic (i.e., nonspecific) patterns of morphol. damage in growing (<6 day old) or mature (6-15 day old) cultures in both a concn.-dependent and cell type-specific manner. In addn., exposure to some toxicants consistently reduced the staining intensity for glial fibrillary acidic protein in the astrocyte

carpet prior to the appearance of structural damage. These data indicate that histopathol. endpoints, including methods for neural-specific markers, represent potentially valuable criteria for in vitro assessments of neurotoxicity.

5
Bird KT. COMPARISONS OF HERBICIDE TOXICITY USING IN VITRO CULTURES OF MYRIOPHYLLUM SPICATUM. Journal of Aquatic Plant Management 1993;31(Jan Spec Ed):43-45.

In vitro cultures of the aquatic plant Myriophyllum spicatum L. were used to determine the effects of the herbicides 2,4-D (dichlorophenoxyacetic acid), atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) and glyphosate (N-(phosphonomethyl) glycine) and the leaf defoliant thidiazuron (N-phenyl-N'-1,2,3-thiadiazol-5-yl-urea) on plant development. The developmental response measured percent reduction of new branches relative to controls. Linear regressions of percent branch number reduction (BNR) as a function of the log (toxin concentration+1) were highly correlated and statistically significant. The plant growth regulator 2,4-D had the greatest effects on development of branches with a 50% BNR of 0.04 mg/l, followed by glyphosate (1.6 mg/l), atrazine (3.7 mg/l), and thidiazuron (9.8 mg/l). The short 5-day time period required for these assays and ability to determine dose-response relationships supports the use of in vitro culture of Myriophylium spicatum as a bioassay system.

6
Gardner DK, Lane M, Spitzer A, Batt PA. ENHANCED RATES OF CLEAVAGE AND DEVELOPMENT FOR SHEEP ZYGOTES CULTURED TO THE BLASTOCYST STAGE IN VITRO IN THE ABSENCE OF SERUM AND SOMATIC CELLS: AMINO ACIDS, VITAMINS, AND CULTURING EMBRYOS IN GROUPS STIMULATE DEVELOPMENT. Biology of Reproduction 1994; 50(2):390-400.

The aim of this study was to develop a serum-free culture system that could support high levels of cleavage and blastocyst formation from sheep zygotes developed in vitro. To this end, we investigated the effects on sheep zygote development of amino acids, ammonium, vitamins, and culture of embryos in groups in Synthetic Oviduct Fluid (SOF) medium supplemented with BSA (32 mg/ml). The inclusion of amino acids in the culture medium had no effect on the percentage of embryos arrested at the 8-16-cell stage when embryos were cultured singly in the same drop of medium for 6 days (43% in SOF; 41% in SOF + amino acids). However, in medium containing all Eagle's amino acids, replacing the culture medium every 48 h to alleviate ammonium toxicity significantly decreased the number of arrested embryos (6%; p <

0.05) and significantly increased blastocyst cell number (52 cells in SOF; 105 cells in SOF + amino acids; $p < 0.01$) and the number of embryos developing to the blastocyst stage (29% in SOF; 67% in SOF + amino acids; $p < 0.05$). When the medium was renewed every 48 h, nonessential amino acids and glutamine also significantly decreased the number of arrested embryos ($p < 0.05$). Culturing embryos singly or in groups in SOF medium with all Eagle's amino acids that was renewed every 48 h resulted in significant increases in blastocyst hatching and mean cell number (47%, 31%, and 79%; 105, 136, and 173 cells for embryos cultured singly, in groups of 2, and in groups of 4, respectively). After culture in groups of 4, blastocyst cell numbers were equivalent to in vivo-developed controls (160 cells) and significantly greater than those developed in serum (103 cells; $p < 0.01$). Analysis of blastocyst metabolism, expressed on a per-cell basis, revealed that amino acids did not affect either glucose uptake or lactate production, whereas the addition of amino acids and vitamins resulted in a significant increase in both parameters ($p < 0.01$). A similar response was observed in serum-derived blastocytes. Ammonium production by sheep blastocysts after culture in the presence of amino acids was significantly greater than that produced by mouse blastocysts, indirect evidence that ruminant embryos utilize amino acids to a greater extent than do rodent embryos. In summary, the data demonstrate that 1) sheep embryo development in culture is impaired by ammonium; 2) maximal development to the blastocyst occurs in the presence of all Eagle's amino acids; 3) Eagle's amino acids significantly increase blastocyst formation, hatching, and cell number, but do not affect glucose uptake or lactate production per cell; 4) vitamins do not affect embryo morphology but do significantly increase glucose uptake and lactate production per cell; 5) sheep embryos produce a factor(s) that stimulates their development in culture. The culture of sheep zygotes in groups of 4 in the presence of Eagle's amino acids for 6 days, with the medium renewed every 48 h, resulted in 95% blastocyst development, 79% hatching rate, and a blastocyst cell number of 173. It is therefore possible to obtain in vitro cleavage rate equivalent to those in vivo, in the absence of somatic cells in a serum-free culture system.

7

O'Reilly S, Mothersill C, Seymour CB, Riches A, Bryant P. DETERMINATION OF THE NUMBER OF SURVIVING CELLS AVAILABLE FOR TRANSFORMATION FOLLOWING IRRADIATION. Annual Meeting of the Association for Radiation Research, Guildford, England, UK, July 12-15, 1993. International Journal of Radiation Biology 1994;65(1):146.

No abstract.

8
Tiffany-Castiglioni E. CELL CULTURE MODELS FOR LEAD TOXICITY IN NEURONAL AND GLIAL CELLS. Neurotoxicology 1993;14(4):513-36. (158 REFS)

Two goals of lead (Pb) neurotoxicity research are to identify molecular and cellular alterations that underlie behavioral deficits and to define mechanisms of Pb uptake and tolerance in cells that accumulate Pb. Cell and tissue cultures are practical tools with which to pursue these goals, offering such advantages over in vivo methods as defined cell types, an extracellular environment that can be precisely manipulated, and direct observation. On the other hand, toxicity studies with cultured cells also present new challenges of design and interpretation. If a living vertebrate is like an orchestra playing a Beethoven symphony, then tissue culture is like two of the violinists playing their part alone. Historically, Pb toxicity studies with cell and tissue culture can be divided into an exploratory phase, an expansion phase, and a newly emerging intensification phase. In the exploratory phase, gross cytotoxic effects from massive Pb exposure (50-500 microM) were characterized. The collective data suggest differential sensitivity to Pb toxicity among various types of cultured neural cells, ranked as follows from most to least sensitive: myelinating cells, neurons, and astroglia. In addition, astroglia were shown to take up and store large amounts of Pb intracellularly, a phenomenon resembling the Pb-sequestering ability hypothesized for mature astroglia in vivo. The mechanisms of Pb entry may involve an anion exchanger, Ca2+ channels, or some other transport process. Three ingrained problems concerning the use of cell cultures began to emerge: appropriate dose regimens, biologically relevant forms of Pb (i.e. ionized or complexed with other molecules), and suitable measurements of Pb effects. These problems received scrutiny in the expansion phase, during which subcellular targets of Pb-induced damage were examined, specifically membranes, enzymes, and Ca-mediated cellular processes. Investigators attempted to define a biologically relevant dose regimen in vitro, as well as a threshold dose below which Pb had no biological effect. Effects of Pb at nanomolar concentrations in intact cells and tissue homogenates stimulated the metamorphosis of Pb toxicity studies in cell culture into a new phase, the intensification phase. Alterations in discrete molecular targets, particularly those effects in the cell that may be metabolically amplified, will be a major focus of this phase. Critical molecular targets for Pb-induced injury appear to be present during neuritogenesis and/or synaptogenesis. With the availability of cell culture models for neurite extension and synapse formation, this area may be another focus for innovative Pb neurotoxicity research.

9
Bigatti MP, Lamberti L, Rizzi FP, Cannas M, Allasia G. IN VITRO MICRONUCLEUS INDUCTION BY POLYMETHYL METHACRYLATE BONE CEMENT IN CULTURED HUMAN LYMPHOCYTES. Mutat Res 1994;321(3):133-7.

Human lymphocytes cultured in vitro were used to assess the ability of polymethyl methacrylate (PMMA), currently used in orthopaedic surgery as bone cement, to induce micronuclei in binucleated cells. The results of the study show a significant increase in the micronucleus frequency in treated cultures and therefore the genotoxic effect of PMMA bone cement or its ingredients (methyl methacrylate, dimethyl para-toluidine and hydroquinone) usually present in self-curing methacrylate bone cement and released in small quantities after polymerisation. This effect is evident during the stage immediately after the polymerisation process, and after a certain period of time (5 days in our experimental model).

CYTOTOXICITY
10
Marinovich M, Viviani B, Galli CL. THE PREDOMINANT ROLE OF SURFACTANTS IN THE MODULATION OF TOXICITY OF DETERGENT PRODUCTS: AN IN VITRO ANALYSIS OF SHAMPOOS. Toxicol in Vitro 1994; 8(1):91-8.

Twenty shampoos and their component surfactants were studied in vitro using a murine epidermal cell line (HEL/30). Lactate dehydrogenase (LDH) leakage into the cell medium and the inhibition of protein synthesis were evaluated as markers of cytotoxicity after 2 h of exposure. The coeffs. of correlation were -0.83 and -0.69 for comparisons between total surfactant content and inhibition of protein synthesis and leakage of LDH, resp. There was an inverse relation between the inhibition of protein synthesis and the amphoteric surfactant content of the shampoo. An excellent correlation (r = 0.94) was found between LDH leakage values and scores from eye irritation tests in vivo on seven of the shampoos. The results obtained suggest that this model allows quant. evaluation of the cytotoxicity of detergent products (shampoos) and of the contribution of their major components (surfactants) to that toxicity.

11
Wilhelm KP, Samblebe M, Siegers CP. QUANTITATIVE IN VITRO ASSESSMENT OF N-ALKYL SULFATE-INDUCED CYTOTOXICITY IN HUMAN KERATINOCYTES (HaCaT). COMPARISON WITH IN VIVO HUMAN IRRITATION TESTS. Br J Dermatol 1994;130(1):18-23.

A spontaneously immortalized human keratinocyte line, HaCaT, was used as an in vitro model to predict the cutaneous irritation of

anionic surfactants. For this purpose, a no. of sodium salts of N-alkyl sulfates with hydrocarbon chain lengths varying between C8 and C16 were studied for possible cytotoxic effects. The endpoints used to assess toxicity were uptake of the vital dye neutral red (NR) and cell morphol. criteria 24 h after dosing. A linear proportionality between keratinocyte no. and NR uptake was established. All tested surfactants had cytotoxic effects as demonstrated by a decreased NR uptake, which showed a clear dose-response relationship. Concns. resulting in 50% inhibition of NR uptake (IC-50) ranged from 0.cntdot.15 mmol (sodium lauryl sulfate, C12) to 1.cntdot.23 mmol (sodium octyl sulfate, C8). The in vitro cytotoxicity data were highly reproducible when the test was repeated after several weeks. The cytotoxicity data from these assays were compared with the irritant responses (as evaluated by measurement of erythema and transepidermal water loss) obtained after 24 h application of the same compds. (300 muL of 20 mmol aq. soln.) to the volar forearm of human volunteers. There were significant linear correlations between the IC-50 values and both barrier damage (transepidermal water loss) and erythema (as evaluated by skin color reflectance measurements). For the test substances, however, the sensitivity of the in vitro system was between 10 and 100 times higher than that obsd. in human skin in vivo. This quant. difference can largely be ascribed to the effective permeability barrier of normal human skin in vivo, which protects living keratinocytes from the cytotoxic effects ofsurfactant mols. The results indicate that normal human keratinocytes in culture are a promising screening method for predicting the irritation potential of anionic surfactants. Confirmation, however, has still to be obtained by appropriate in vivo testing in human volunteers.

12
Weiss MT, Sawyer TW. CYTOTOXICITY OF THE MEIC TEST CHEMICALS IN PRIMARY NEURON CLUTURES. Toxicol in Vitro 1993;7(5):653-67.

Fifty Multicenter Evaluation of In Vitro Cytotoxicity (MEIC) test chems. were assayed for cytotoxicity in primary cultures of chick embryo forebrain neurons using the MTT and neutral red assays. The neutral red assay was consistently more sensitive to chem. toxicity; however, both assays were equally predictive when compared with in vivo toxicity data obtained from the Registry of Toxic Effects of Chem. Substances. High correlations were obtained when comparing the in vitro data with i.p. rodent toxicity data, but these correlations decreased significantly when oral toxicity data were used. The predictive value of the in vitro data for oral human toxicity was generally poor, but comparable with its value in predicting oral rodent toxicity. In a limited study with 10 of the MEIC test chems., the cytotoxicity of some compds. was dependent on the degree of differentiation of

the neuron cultures, suggesting that this culture system may not only be sensitive to the basal cytotoxicity of chems., but also to toxic effects specific to the specialized differentiated functions of the central nervous system.

13
Kohn J, Durham HD. S9 LIVER FRACTION IS CYTOTOXIC TO NEURONS IN DISSOCIATED CULTURE. Neurotoxicology 1993; 14(4):381-6.

The suitability of the S9 activation system for long-term neurotoxicity studies in vitro was investigated in dissocd. cultures of murine spinal cord-dorsal root ganglia. Exposure to amts. of S9 greater than 0.07 mg S9 protein/mL of culture medium for 4 days or longer was cytotoxic to all types of neurons in the cultures. Nonneuronal cells tolerated higher exposures, but contained numerous cytoplasmic inclusions when 0.35 mg S9 protein was included in the medium. It was demonstrated that cytotoxicity was caused by the particulate, microsomal fraction of S9. It is concluded that direct incorporation of S9 fraction in the growth medium (0.07 mg S9 protein/mL or greater) is not a suitable method of generating metabolites in dissocd. cultures of central nervous system when several days are required to elicit a biol. response. Cytotoxicity can be prevented by using tissue culture inserts to sep. cultured cells from S9 particulate fraction by a microporous membrane.

14
Dierickx PJ. COMPARISON BETWEEN FISH LETHALITY DATA AND THE IN VITRO CYTOTOXICITY OF LIPOPHILIC SOLVENTS TO CULTURED FISH CELLS IN A TWO-COMPARTMENT MODEL. Chemosphere 1993;27(8):1511-18.

Cytotoxicity testing of non-hydrosol. chems. offers a major problem because cells are always cultured in aq. media. An adaptation of the two-compartment model of Boue-Grabot et al. (1992) is reported here. The cytotoxicity of 19 lipophilic solvents was measured on cultured FHM (fathead minnow fish) cells. The FHM cells were seeded in transwells on a 0.4 mum pore membrane (upper compartment) which are placed in the wells of a 24 well culture plate (lower compartment). The transwells were then placed in wells contg. the test chem., solubilized in paraffin. After 24 h, the total protein content was measured. The relative toxicity is expressed by the EC50. This is the concn. of test chem. in the lower compartment required to induce a 50% inhibition of the total protein content in the upper compartment. No linear correlation was obtained between the EC50 of the lipophilic solvents and the in vivo fish lethality data obtained in golden orfe by Juhnke and Ludemann (1978). Nevertheless, this method allows the ranking of quant. cytotoxicity data of lipophilic chems. towards cultured fish cells.

15
Silvestrini R, Zaffaroni N, Orlandi L, Oriana S. IN VITRO CYTOTOXIC ACTIVITY OF TAXOL AND TAXOTERE ON PRIMARY CULTURES AND ESTABLISHED CELL LINES OF HUMAN OVARIAN CANCER. Stem Cells (Dayton) 1993;11(6):528-35.

The activity of taxol and taxotere was evaluated on three established cell lines and 19 primary cultures of human ovarian cancers and compared with that of cisplatin and doxorubicin. The cytotoxic activity of the different drugs was assessed with a clonogenic assay in cell lines and with a proliferative assay (based on [3H]thymidine [3H-dT] incorporation of cell grown in double-layer agarose for four days) in primary tumor cultures. The two assays run in parallel on the OVCA432 cell line provided similar ranking of activity for all the drugs. Taxotere was more cytotoxic than taxol in two cell lines and showed the same degree of activity in one cell line. Moreover, the two drugs were more cytotoxic than cisplatin and doxorubicin in all cell lines. In primary cultures both taxol and taxotere were less active than cisplatin and doxorubicin. An activity by at least one of these two compds. was seen in 9 of 19 cases. Taxol was more frequently active than taxotere and generally more potent. A direct relationship was obsd. between the proliferative activity of the tumor cell population and response to taxol and /or taxotere. In fact, cell lines that were highly sensitive to taxol and taxotere displayed 3H-dT labeling index (LI) values much higher than those obsd. in primary cultures (30% to 45% vs. 0.2% to 12.6%). Again, primary cultures sensitive to taxol and/or taxotere were characterized by a median 3H-T LI value about three times higher than that obsd. in resistant cultures (8.0% vs. 2.6%).

16
Pessina A, Gribaldo L, Mineo E, Neri MG. IN VITRO SHORT-TERM AND LONG-TERM CYTOTOXICITY OF FLUOROQUINOLONES ON MURINE CELL LINES. Indian J Exp Biol 1994;32(2):113-18.

Short-term and long-term cytotoxicity of four fluoroquinolones (ciprofloxacin, rufloxacin, ofloxacin, lomefloxacin) on 7 established murine cell lines (WEHI-3B, L1210, EL4, P388D1, 32DC13, L929, SR-4987) by microtiter MTT assay have been studied. In short-term cytotoxic test (24 h), cell lines with a high proliferating cell rate (as leukemias) showed a greater sensitivity to quinolones than other cell lines. In long-term cytotoxic text (7 days) no different sensitivity was obsd. among the cell lines. In short- term test ciprofloxacin and rufloxacin were more toxic than lomefloxacin and ofloxacin whereas in the long-term test the activity of the four quinlones was similar. Ratio between IC50 on cell lines and MIC50 against gram neg.

bacteria evidenced remarkable differences on short-term or long-term cytotoxic tests were considered. The results confirm toxic activity of quinolones on mammalian cells evidencing that the sensitivity to quinolones, in short-term cytotoxic test, correlates with the doubling time of cell population. The results further suggest that long-term cytotoxic tests measure better the antiproliferating activity of quinolones providing a more powerful assay to investigate their in vitro toxicity.

17
Jover R, Ponsoda X, Castell JV, Gomez-Lechon MJ. ACUTE CYTOTOXICITY OF TEN CHEMICALS IN HUMAN AND RAT CULTURED HEPATOCYTES AND IN CELL LINES: CORRELATION BETWEEN IN VITRO DATA AND HUMAN LETHAL CONCENTRATIONS. Toxicol in Vitro 1994;8(1):47-54.

The cytotoxicity of ten chems. from the MEIC list (nos 11-20) was evaluated in human and rat cultured hepatocytes and in two established cell lines (HepG2 and 3T3) according to the Multicentre Evaluation of In vitro Cytotoxicity program organized by the Scandinavian Society of Cell Toxicol. The lactate dehydrogenase intracellular activity and the MTT test were used as endpoints of cytotoxicity after 24 h of exposure to the chems. Sodium chloride and lithium sulfate were the least cytotoxic compds. in all of the cellular systems (IC50, 25-150 mM). The eight remaining chems. (1,1,1-trichloroethane, phenol, sodium fluoride, malathion, 2,4-dichlorophenoxyacetic acid, xylene, nicotine and potassium cyanide) showed a similar cytotoxic potential in the four in vitro systems in a narrow range of concns. (IC50, 1-30 mM). The data suggest that these ten chems. have a basal cytotoxic effect common to the four in vitro systems, and probably none of these compds. could be considered either hepatotoxic or to exert species-specific toxicity. The correlation between in vitro data and human lethal blood concns. showed a relatively low predictability for the toxicity of six

compds. with important lethal effects on the CNS. The predictability of the in vitro systems was similar to that of in vivo rodent tests (LD50) only when low cytotoxic concns. (IC10) were used for the correlation.

18
Parent-Massin D, Thouvenot D, Rio B, Riche C. LINDANE HAEMATOTOXICITY CONFIRMED BY IN VITRO TESTS ON HUMAN AND RAT PROGENITORS. Human & Experimental Toxicology 1994; 13(2):103-106.

Blood dyscrasias such as aplastic anemia and leukopenia are described following the use of lindane for agricultural purposes

or against ectoparasites in animal and human health. In order to determine the involvement of lindane in these effects, an in vitro model of haematotoxicity evaluation has been used. Culture of haematopoietic progenitors, Colony Forming Unit-Granulocyte and Macrophage (CFU-GM), have been performed in the presence of lindane with increasing concentrations. Results showed th lindane

was cytotoxic for human progenitors. There were one thousand times more sensitive to the lindane than rat CFU-GM. This cytotoxicity was observed with lindane concentration similar to those measured in human blood in cases of acute intoxication and in fat tissues of exposed populations.

19
Abdul-Hussain SK, Levin P, Acosta D. CYTOTOXICITY INDUCED BY TWEEN-20 A NONIONIC SURFACTANT IN PRIMARY KERATINOCYTE CULTURES FROM NEONATAL RAT SKIN. Experimental Biology 94, Parts I and II, Anaheim, California, USA, April 24-28, 1994. FASEB Journal 1994;

8(4-5):A648.

No abstract.

20
Katsnel'son BA, Privalova LI, Sharapova NE, Kislitsina NS. IMPORTANCE OF THE SYSTEM OF SHORT-TERM CYTOTOXICITY TESTS FOR HEALTH-RELATED CHARACTERIZATION OF INDUSTRIAL AEROSOLS. Meditsina Truda I Promyshlennaya Ekologiya 1993;0(1):2-7.

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

DEVELOPMENTAL TOXICITY
21
Odio MR, Azri-Meehan S, Robison SH, Kraus AL. EVALUATION OF SUBCHRONIC (13 WEEK), REPRODUCTIVE, AND IN VITRO GENETIC TOXICITY POTENTIAL OF 2-ETHYLHEXYL-2-CYANO-3,3-DIPHENYL ACRYLATE (OCTOCRYLENE). Fundam Appl Toxicol 1994;22(3):355-68.

The present studies evaluated subchronic toxicity, developmental toxicity, and in vitro genotoxic potential of Octocrylene. In the subchronic study, male and female New Zealand white (NZW) rabbit treated topically with concns. of Octocrylene up to 534 mg/kg/day for 13 wk showed slight to moderate dose-dependent skin irritation that correlated pos. with a mild depression in body wt. gain. Lack of assocd. histopathol. or clin. hematol. abnormalities suggested that the body wt. effect probably reflected a nonspecific response to topical irritation. In percutaneous developmental toxicity studies, NZW does were treated topically with Octocrylene at levels up to 267 mg/kg/day on Days 6 through 18 of gestation. Body wt. gain, food consumption, and all maternal, productive, and offspring parameters evaluated were comparable between Octocrylene-treated and control animals. In the oral developmental toxicity assay,

female CD-1 mice received oral doses of Octocrylene up to 1000 mg/kg/day on days 8-12 of gestation. No evidence of maternal or developmental toxicity was seen at any dose tested. Genotoxicity was evaluated in vitro using the Chinese hamster ovary cell assay to assess clastogeniticity and the mouse lymphoma cell assay to assess forward gene mutations. Octocrylene did not induce any significant increase in genotoxicity. This evaluation of toxicol. potential supports the use of Octocrylene as a human photoprotectant.

22
Piersma AH, Haakmat AS, Hagenaars AM. IN VITRO ASSAYS FOR THE DEVELOPMENTAL TOXICITY OF XENOBIOTIC COMPOUNDS USING DIFFERENTIATING EMBRYONAL CARCINOMA CELLS IN CULTURE. Toxicol in Vitro 1993;7(5):615-21.
Murine embryonal carcinoma (EC) cells, which resemble the undifferentiated cells of the epiblast in the blastocyst, were used to establish 2 in vitro assays for developmental toxicants. The target processes in the assays are the differentiation of EC cells into endodermal and mesodermal derivs., resp. These processes were selected because they are crucial and specific for embryogenesis, and also because EC cell differentiation has been shown to be sensitive to various compds. that are teratogenic in vivo. The usefulness of the assays was studied with 5 pairs of xenobiotic compds., with chem. analogy and different in vivo teratogenicity within each pair. Results for imidazoles and pyridines correlated well with known effectiveness in vivo; however, this was not the case for phthalates, sulfonamides, and xanthines, which for the latter is explicable in terms of mechanisms of action in vivo. Validation of the assays for classes of related compds. will det. their usefulness as a screen for each class, depending on the mechanism of action and phys. properties. In this way, in vitro assays

will contribute to the refinement of testing, in addn. to the more efficient use of lab. animals.

GENOTOXICITY

23
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 obsd. for the fly ash ext. from peat combustion, an intermediate induction was found for the fly ash ext. from biosludge combustion, and a strong induction was detected for natural peat ext. 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 addn. to conventional genotoxicity tests, which may fail to detect potentially harmful compds./mixts.

24
Seemayer NH, Hornberg C, Hadnagy W. COMPARATIVE GENOTOXICITY TESTING OF AIRBORNE PARTICULATES USING RODENT TRACHEAL EPITHELIAL CELLS AND HUMAN LYMPHOCYTES IN VITRO. Fourth European Meeting of Envirnomental Hygiene, Wageningen, Netherlands, June 9-11, 1993. Zentralblatt fuer Hygiene und Umweltmedizin 1994;195(2):174.

No abstract.

25
Schmezer P, Kuchenmeister F, Klein RG. GENOTOXIC ACTIVITY OF HEAVY METAL COMPOUNDS IN PRIMARY CELLS OF RESPIRATORY TISSUES. Fourth European Meeting of Envirnomental Hygiene, Wageningen, Netherlands, June 9-11, 1993. Zentralblatt fuer Hygiene und Umweltmedizin 1994;195(2):173.

No abstract.

26
Perchermeier M, Kiefer F, Reuter U, Weibel FJ. ESTABLISHMENT OF MAMMALIAN CELL LINES FOR TESTING THE CYTO- AND GENOTOXICITY OF NITROAROMATIC COMPOUNDS. Fourth European Meeting of Envirnomental Hygiene, Wageningen, Netherlands, June 9-11, 1993. Zentralblatt fuer Hygiene und Umweltmedizin 1994;195(2):167.

No abstract.

27
Madle S, Dean SW,Andrae U, Brambilla G, Burlinson B, Doolittle J, Furihata C, Hertner T, McQueen CA, Mori H. RECOMMENDATIONS FOR THE PERFORMANCE OF UDS TESTS IN VITRO AND IN VIVO. Mutat Res 1994;312(3):263-85. (81 REFS)

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 UDSguideline), 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 analysed by means of autoradiography (AR) or liquid 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 analysed individually. Since a specific cell type was recommended by the WG, methodological 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 experiments in the event of a clear-cut negative result, the majority view was that confirmation by a second (normally not identical) experiment 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 recognised that nuclear and cytoplasmic grains result from different biological processes. Since grain counts are influenced by a number of methodological parameters, no global threshold NNG value can be recommended for discrimination of positive 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 methodology 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.(Abstract truncated at 400 Words)

28
Tavares DC, Takahashi CS. EVALUATION OF THE GENOTOXIC POTENTIAL OF THE ALKALOID BOLDINE IN MAMMALIAN CELL SYSTEMS IN VITRO AND IN VIVO. Mutat Res 1994;321(3):139-45.

Boldine is an alkaloid present in Peumus boldus (popularly called "boldo-do-chile" in Brazil) which has healing properties and is used for the treatment of gastrointestinal disorders. The possible clastogenic effect of the drug was tested in vitro on human peripheral blood lymphocytes by evaluating the induction of chromosome aberrations and sister-chromatid exchanges (SCEs). Cultures from different individuals were treated with boldine at concentrations of 10, 20 and 40 micrograms/ml of culture medium. The effect of the alkaloid was also tested in an in vivo assay using BALB/c mouse bone marrow cells. Boldine was administered to the animals by gavage at the concentrations of 225, 450 and 900 mg/kg body weight. Under the conditions used, boldine did not induce a statistically significant increase in the frequency of chromosome aberrations or SCEs in either test system.

29
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(3):11-3.

The lymphocyte micronucleus assay was used to measure the average frequency of micronuclei in a population and thus assess genotoxic effects. Data from 174 persons give an average value of 16.4 +/- 7.3, and a slight age-dependence was observed. To detect combined environmental mutagen injuries the micronucleus assay was used to study the effects of metal compounds. Cadmium ions increased the micronucleus frequency linearly after incubation with whole blood in vitro with 10(6)-10(-3) M concentrations for 30 min. Similarly, a linear increase in micronucleus frequency was detected with 10(-3)-10(-1) M mercury ions. Concerning the biological effect of selenium, it was found that neither sodium selenite nor selenium dioxide induced increases at concentrations of 10(-7)-10(-6) M; 10(-5) M caused a slight increase; 10(-4) M, however, destroyed the cells. These results suggest that the human lymphocyte micronucleus test can be used to assess

genotoxic injuries due to environmental effects in human lymphocytes.

30
Fenech M. THE CYTOKINESIS-BLOCK MICRONUCLEUS TECHNIQUE AND ITS APPLICATION TO GENOTOXICITY STUDIES IN HUMAN POPULATIONS. Environ Health Perspect 1993;101(suppl 3):101-7. (51 REFS)

The development of the cytokinesis-block (CB) technique has made the human lymphocyte micronucleus assay (MN) a reliable and precise method for assessing chromosome damage. Recent studies in our laboratory have confirmed that this method is a sensitive indicator of in vivo radiation exposure in patients undergoing fractionated partial-body radiotherapy and rodents exposed to uniform whole-body irradiation, thus supporting the application of the cytokinesis-block micronucleus (CBMN) assay for biological dosimetry. To further define the use of this assay in biomonitoring, we have also undertaken extensive studies to determine the spontaneous level of MN in normal human populations and its relationship to various lifestyle factors. During the past year, we have also developed a new variation to the CBMN assay that enables the conversion of excision-repairable lesions to MN within one cell-cycle using cytosine arabinoside. With this method the slope of the in vitro dose-response curves was increased by a factor of 1.8 for X-rays, 10.3 for ultraviolet (254 nm) radiation, and approximately 40-fold for methylnitrosourea. Consequently, the CBMN assay can now be used not only to measure whole chromosome loss or chromosome breaks but also excision repair events. The versatility and simplicity of the CBMN assay together with new developments in automation should enable its successful application in monitoring exposed populations as well as identifying mutagen-sensitive individuals within a population.

31
Galloway SM, Aardema MJ, Ishidate M Jr, Ivett JL, Kirkland DJ, Morita T, Mosesso P, Sofuni T. REPORT FROM WORKING GROUP ON IN VITRO TEST FOR CHROMOSOMAL ABERRATIONS. Mutat Res 1994; 312(3):241-61. (48 REFS)

The following summary represents a consensus of the working group except where noted. The items discussed are listed in the order in which they appear in the OECD guideline (473) for easy reference. Metabolic activation. S9 from animals induced either with Aroclor 1254 or with the combination of phenobarbital with beta-nephthoflavone is acceptable, and other systems could be used with suitable justification. Exposure concentrations. the upper limit of testing should be 10 mM (or 5 mg/ml where molecular weight is not known or mixtures being tested),

whichever is lower. Where this limit is inappropriate the investigator should give detailed justification of the choice of top concentration. Cytotoxicity should be measured not only in range-finding tests but also concurrently with the assay for chromosomal aberrations. Cytotoxicity should be assessed by measurements of cell growth such as cell counts or confluence estimation. Mitotic index data alone are not a sufficient measure of cytotoxicity, except in the case of blood cultures for which other methods are impractical. Cytotoxicity at the top dose should be greater than 50% of concurrent negative/solvent controls, if this can be achieved without exceeding a concentration limit of 10 mM or 5 mg/ml. There should be at least three concentrations scored for aberrations (each with and without S9), covering a toxicity range down to a concentration giving little or no cytotoxicity. This will usually mean that the concentration scored with be quite closely spaced. It was not possible to reach a consensus on the issue of solubility limits. The group did not agree on whether (a) solubility rather than cytotoxicity should be the limiting factor, such that only one top dose with evident precipitate should be scored even if toxicity is not observed, or (b) several concentrations with evident precipitate should be scored for aberrations if this were necessary to obtain cytotoxicity. It was agreed that evidence of precipitation should be determined in the final culture medium. Controls. Concurrent positive controls are required but the working group thought it inappropriate to specify the control chemicals or the degree of response that should be obtained, leaving it up to the test laboratory to demonstrate that the system was working adequately based on historical data within the laboratory. It is not necessary to include both negative and solvent controls concurrently with the aberration test; solvent controls alone are acceptable provided that the laboratory has data to demonstrate that there is no effect of the solvent on baseline values. Preparation of cultures. (Abstract Truncated at 400 words.)

32
Kirkland DJ. INTERNATIONAL WORKSHOP ON STANDARDIZATION OF GENOTOXICITY TEST PROCEDURES. REPORT OF THE IN VITRO SUB-GROUP. Mutat Res 1994;312(3):211-5 (5 REFS)

No abstract.

HEPATOTOXICITY
33
Lubinski J, Flint OP, Durham SK. ANTI-HIV NUCLEOSIDE ANALOGS: IN VITRO TOXICITY STUDY USING RAT, RABBIT, DOG, AND MONKEY HEPATOCYTES IN PRIMARY CULTURE. In Vitro Toxicol 1993; 6(4):279-89.

Hepatotoxicity is an infrequent occurrence in patients receiving 2',3'-dideoxyinosine (ddI, didanosine). To det. the intrinsic hepatotoxic potential of ddI, the in vitro toxicity of ddI and 3 other nucleoside analogs, 2'-fluoro-2',3'-dideoxyinosine (FddI), 2'-fluoro-2',3'-dideoxyadenosine (FddA), and 3'-azido-2',3'-dideoxythymidine (AZT, zidovudine), was compared using hepatocytes from several species in primary culture. The effect of cytochrome P 450 content on nucleoside hepatotoxicity was also evaluated. The endpoints assessed after a 24-h exposure to test article in vitro were cell membrane integrity (lactate dehydrogenase [LDH] release), cell viability (MTT metab. by mitochondrial dehydrogenases), protein synthesis (incorporation of [3H]leucine), and energy status (intracellular ATP content). The concns. of test agent inhibiting an endpoint by 50% control values (IC50) for ddI, FddI, and AZT were all >2880 mug/mL and generally greater or equiv. to the IC50 measured for the neg. control, penicillamine. These IC50 values were considered too high to be biol. relevant to any potential hepatotoxicity in vivo. Similar results were obtained from hepatocytes prepd. from all species tested; however, monkey hepatocytes were relatively more sensitive to ddI, FddI, and AZT, while dog and rabbit hepatocytes were more sensitive to FddA. Nucleoside analog-induced toxicity in vitro did not change markedly when rat hepatocytes with different starting levels of cytochrome P 450 were used. Thus, the hepatotoxic potency of nucleoside analogs did not depend on the level of drug-metabolizing enzymes in hepatocytes in primary culture.

34
Fisher RL, Gandolfi AJ, Sipes IG, Brendel K. CULTURE MEDIUM COMPOSITION AFFECTS THE RELATIVE TOXICITIES OF CHLOROBENZENES IN RAT LIVER SLICES AND THE ISOLATED PERFUSED LIVER. Drug and Chemical Toxicology an International Journal for Rapid Communication 1993;16(4):321-339.

The effects of different media composition on the hepato-toxicity produced by monochlorobenzene MCB), 1,2-dichlorobenzene (1,2-DCB), 1,3-dichlorobenzene (1,3-DCB) and 1,4-dichlorobenzene (1,4-DCB) were examined in two different in vitro systems. The toxicity of these chlorobenzenes was investigated in the perfused rat liver and liver slices using Krebs-Henseleit buffer. Significant differences between the chlorobenzenes were apparent in the perfused liver but not in the tissue slices. However, a dose and time related response of rat liver slices to the chlorobenzenes was observed. Partial amelioration of the chlorobenzene toxicity was observed when the Krebs-Henseleit buffer was supplemented with vitamins, amino acids, and/or bovine serum albumin. 1,2-DCB and 1,3-DCB toxicity was affected by amino acids and vitamins. The toxicity produced

by 1,4-DCB was suppressed by amino acids, vitamins and 1% BSA. MCB hepatoxicity could only be suppressed by 1% BSA. This data suggests that tissue culture media composition plays a major role in the hepatotoxicity of the chlorobenzenes.

IMMUNOTOXICITY
35
Reid LL, Hastings KL, Gandolfi AJ, Van Ert M. USE OF STAPHYLOCOCCAL ENTEROTOXIN A-INDUCED LYMPHOPROLIFERATION AND INTERLEUKIN 2 PRODUCTION AS INDICATORS OF IMMUNOTOXICITY. Drug Chem Toxicol 1994;17(1):1-14.

Suppression of mitogen-induced splenocyte lymphoproliferation and interleukin 2 (IL-2) production can be used as indicators of immunotoxicity. Staphylococcal enterotoxin A (SEA) is both a potent mitogen and the most potent in vitro inducer of IL-2 production that has been described. An in vitro system was used to measure impairment of SEA-induced lymphoproliferation and IL-2 production using splenocytes from female C57BL/6 mice dosed with either cyclosporin A (30 mg/kg/day, 14 days), benzene (220, 440, or 880 mg/kg/day, 14 days), or vehicle. Splenocytes were stimulated with either concanavalin A (con A) or SEA. Benzene- and cyclosporin A-treated mice demonstrated significant decreases in splenocyte proliferation. IL-2 production was determined by incubating splenocyte culture supernatants with IL-2 dependent cytotoxic T-cells (CTLL-2), pulsing with 3H-thymidine, and determining amount of incorporated label. Cell proliferation and IL-2 production were inhibited by both benzene and cyclosporin A, effects more clearly demonstrated using SEA than con A. SEA was a superior mitogen compared to con A in the assays evaluated here.

MECHANISMS/TOXICITY
36

Swaffar DS, Ireland CM, Barrows LR. A RAPID MECHANISM-BASED SCREEN TO DETECT POTENTIAL ANTICANCER AGENTS. Anti-Cancer Drugs 1994;5(1):15-23.

Several mutant Chinese hamster ovary (CHO) cell lines have been adapted to the microtiter tetrazolium assay in order to obtain useful mechanistic information relevant to the cytotoxic activity of marine natural products. The sensitivity of a DNA double-strand break repair deficient CHO line, xrs-6, was compared with that of a DNA repair competent CHO line, BR1, to several known drugs. The deficiency of the xrs-6 cells makes them overly sensitive to compds. [e.g. topoisomerase II (topo II) inhibitors] that produce DNA double-strand breaks. Described here is the validation of this unique cellular screen to defect such compds. Those drugs though to produce their effects by the

inhibition of tope II, produced the largest differential cytotoxicity against the mutant CHO pair. Other agents that are known to either produce single-strand breaks, cross-links or to inhibit the synthesis of DNA did not possess appreciably enhanced cytotoxicity to the xrs-6 line. The usefulness of the screen was shown by its ability to detect topo II inhibitory activity in several new marine natural products. This activity was confirmed by an in vitro enzyme inhibition assay. In contrast, the screen predicted a lack of topo II inhibitory activity in some other structurally related marine natural products and this lack of activity was confirmed by an in vitro enzyme inhibition assay.

37
Thompson DC, Perera K, Fisher R, Brendel K. CRESOL ISOMERS: COMPARISON OF TOXIC POTENCY IN RAT LIVER SLICES. Toxic Appl Pharm 1994;125(1):51-58.

A comparison of the toxicity of cresol isomers (o-, M-, P-methylphenol) was carried out using precision-cut liver slices as a test system. AT equimolar concentrations p-cresol was the most toxic isomer. A 5- to 10-fold concentration of either the o- or m-isomers was required to observe the same degree of cell killing as p-cresol. The toxicity of p-cresol was inhibited by the thiol precursor N-acetylcysteine and was enhanced by pretreatment of liver slices with diethyl maleate to deplete glutathione. These treatments, however, had little effect on either o- or m-cresol toxicity. p-Cresol rapidly depleted intracellular glutathione levels, while the o- and m-isomers depleted glutathione to a lesser extent. (14C)p-cresol was metabolized to a reactive intermediate which covalently bound to slice protein and was inhibited by N-acetylcysteine. In microsomal incubations covalent binding of (14C)p-cresol metabolites was also observed. This binding was inhibited by glutathione and resulted in the formation of a glutathione conjugate. In the absence of glutathione, p-hydroxybenzyl alcohol was the major microsomal metabolite formed form p-cresol, but this compound was not toxic to liver slices at a concentration of 2mM. These results also suggest that the mechanism(s) of toxicity of the o- and m-isomers may differ from that of p-cresol.

MUTAGENICITY
38
Waters MD, Stack HF, Jackson MA, Bridges BA. HAZARD IDENTIFICATION: EFFICIENCY OF SHORT-TERM TESTS IN IDENTIFYING GERM CELL MUTAGENS AND PUTATIVE NONGENOTOXIC CARCINOGENS. Environ Health Perspect 1993;101(3):61-72.

For more than a decade, mutagenicity tests have had a clearly defined role in the identification of potential human mutagens

and an ancillary role in the identification of potential human carcinogens. The efficiency of short-term tests in identifying

germ cell mutagens has been examined using a combined data set derived from the U.S. Environmental Protection Agency/ International Agency for Research on Cancer Genetic Activity Profile (EPA/IARC GAP) and EPA Gene-Tox databases. Our review of these data indicates adequate sensitivity of batteries of in vitro short-term mutagenicity tests in identifying germ cell mutagens. The analysis also supports the inclusion of an in vivo assay as suggested in proposed regulatory testing guidelines. In the context of carcinogenicity testing, the ability of short-term bioassays to detect genotoxic or mutagenic carcinogens is well established. Such tests are not considered to be as sensitive to nongenotoxic or nonmutagenic carcinogens. However, analyses presented in this report using the EPA/IARC GAP database demonstrate that many putative nongenotoxic carcinogens that have been adequately tested in short-term genetic bioassays induce gene or chromosomal mutation or aneuploidy. Further investigation should reveal whether the mutagenicity of these agents plays an important mechanistic role in their carcinogenicity.

39
Jones IM, Moore DH, Thomas CB, Thompson CL, Strout CL, Burkhart-Schultz K. FACTORS AFFECTING HPRT MUTANT FREQUENCY IN T-LYMPHOCYTES OF SMOKERS AND NONSMOKERS. Cancer Epidemiol, Biomarkers Prev 1993;2(3):249-60.

The frequency of thioguanine-resistant, hypoxanthine phosphoribosyltransferase-deficient lymphocytes in the peripheral blood of human subjects was used to study the genotoxic effects of smoking. Sixty-two nonsmokers and 58 smokers, aged 19 to 45 yr with av. ages of 30 and 32 yr, resp., and with no other known exposures, were studied using an in vitro assay of the frequency

of mutant lymphocyte clones. Anal. of variance explained 68% of the variation in the mutant frequencies. Mutant frequency was dependent upon lymphocyte cloning efficiency, length of smoking history, age, and interactions between these variables. Four nonsmokers and three smokers had high mutant frequencies that were not explained by these variables. Mutant frequencies were inversely related to lymphocyte cloning efficiencies; the effect was twice as great for smokers as for nonsmokers. The time-dependent effect of smoking dominated, with mutant frequency increasing 10%/yr of smoking as compared with an independent

1%/yr of age. Smoking had a greater effect on young smokers' lymphocytes. Heterogeneity of mutant frequency among both smokers and nonsmokers and its implications for use of lymphocyte

mutation assays as biomarkers are discussed.

40
Mudry MD, Carballo M, Labal de Vinuesa M, Gonzalez Cid M, Larripa I. MUTAGENIC BIOASSAY OF CERTAIN PHARMACOLOGICAL DRUGS: III. METRONIDAZOLE (MTZ). Mutat Res 1994;305(2):127-32.

The genotoxic activity of MTZ was evaluated in vitro with the anaphase-telophase test in a CHO cell line, chromosomal aberration and micronucleus test in lymphocyte cultures, and in vivo using the micronucleus test in mouse bone marrow cells. The in vivo test was performed using clin. trial doses (23, 70 and 160 mg/kg). A significant increase in micronucleated cells ($p < 0.02$) was obsd. in the 3 assayed doses with a linear dose response ($r = 0.91$). In vitro studies showed a significant increase in the percentage of abnormal anaphases ($p < 0.005$), in chromosome aberrations ($p < 0.01$) and in the frequency of micronuclei ($p < 0.02$) at all the concns. assayed (0.1, 1 and 10 mug/mL). These findings demonstrate the clastogenic effect of this drug which should be taken into account considering its wide human consumption.

41
Galloway SM. CHROMOSOME ABERRATIONS INDUCED IN VITRO: MECHANISMS, DELAYED EXPRESSION, AND INTRIGUING QUESTIONS. Environ Mol Mutagen 1994;23(Suppl 24):44-53. (71 REFS)

Chromosome aberrations, including breakage and rearrangement and numerical changes, are important in carcinogenesis, heritable mutations, embryonic loss, and developmental abnormalities. The authors can detect DAN reactive agents in in-vitro chromosome aberration assays, but aberrations are also induced by chems. that do not directly interact with DNA. This article discusses briefly some important aspects of using aberrations in genetic toxicol. testing but concs. 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 the authors to interpret results of chromosome aberration tests and to develop a rational assessment of the degree of human risk implied by a pos. aberration test.

42

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

Three chemicals were selected for mutagenicity testing from a priority list, based on production volume and available mutagenicity data. Propargyl alcohol (PA), 2-nitroaniline (2NA), 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 2NA, were clastogenic in vitro. Both PA and 2NA 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 2NA were found to be in vitro clastogens, they also were tested in the mouse bone-marrow micronucleus assay. 2NA induced a small increase in micronuclei in males but not females. PA did not induce an increase in micronuclei.

43
Kirkland DJ. STATISTICAL EVALUATION OF MUTAGENICITY TEST DATA RECOMMENDATIONS OF THE U.K. ENVIRONMENTAL MUTAGEN SOCIETY. Environmental Health Perspectives 1994;102(SUPPL 1):43-47.

No abstract.

44
Hoke RA, Jones PD, MacCubbin AE, Zabik MJ, Giesy JP. USE OF IN VITRO MICROBIAL ASSAYS OF SEDIMENT EXTRACTS TO DETECT AND QUANTIFY CONTAMINANTS WITH SIMILAR MODES OF ACTION. Chemosphere 1994;28(1):169-181.

Mutagenicity of organic solvent extracts of contaminated sediments from a Great Lakes Area of Concern (AOC) was evaluated with Ames and Mutatox assays. Extract mutagenicity was evaluated with and without S9 metabolic activation in both assays. H4IIE rat cell hepatoma assays also were conducted on the organic solvent extracts of the sediments. Little direct mutagenicity was observed in either the Ames or Mutatox assays, however, a greater number of extracts were observed to be mutagenic after S9 metabolic activation. Although numerous potentially mutagenic compounds exist in sediments from this AOC, polycyclic aromatic hydrocarbons (PAHs) appear to be the likely cause of a major fraction of the observed mutagenicity in the Ames and Mutatox assays. PAHs, as opposed to compounds with a mode of action

similar to 2,3,7,8-TCDD, also appear responsible for the majority of the enzyme induction observed in the H4IIE assay.

45
Gatehouse D, Haworth S, Cebula T, Gocke E, Kier L, Matsushima T, Melcion C, Nohmi T, Ohta T, Venitt S, et al. RECOMMENDATIONS FOR THE PERFORMANCE OF BACTERIAL MUTATION ASSAYS. Mutat Res 1994;312(3):217-33. (43 REFS)

At the International Workshop on the Standardisation of Genotoxicity Test Procedures, in Melbourne (27-28 February 1993), the current international guidelines for the correct conduct of bacterial mutation assays were considered, and the major differences between them were examined. 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 compound should be used in each experiment, and a majority agreement was obtained for 3 plates per dose. The use of 2 plates per dose is acceptable ONLY if the experiment is repeated. It is recommended that the negative controls may consist of solvent control alone provided that historical data are available to demonstrate lack of effect of the solvent in question. Positive control compounds should be included in all experiments, although the nature of these control compounds need not be specified in the guidelines. There was consensus agreement that for non-toxic freely soluble test agents, an upper limit of 5 mg/plate should be tested (5 microliters per plate for liquids). For insoluble 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 negative results in the first test should be further investigated by either conducting a modified repeat (e.g. S9

titration) or by conducting the alternative methodology.

NEUROTOXICITY

46

Atterwill CK, Davenport-Jones J, Goonetilleke S, Johnston H, Purcell W, Thomas SM, West M, Williams S. NEW MODELS FOR THE IN VITRO ASSESSMENT OF NEUROTOXICITY IN THE NERVOUS SYSTEM AND THE PRELIMINARY VALIDATION STAGES OF A TIERED-TEST MODEL. Toxicol in Vitro 1993; 7(5):569-80.

Many cell culture models are available for the in vitro assessment of neurotoxicity. The use of 3 culture types was investigated: neuroblastoma cell lines, primary cultures of rat and chick midbrain, and organotypic Whole brain reaggregate cultures. A tiered system was proposed involving hierarchical testing through 3 layers of different neural complexities. This scheme is currently undergoing validation under the auspices of FRAME/EC using 40 test chems. To det. the performance and suitability of these culture models, studies on selected neurotoxins were performed: ethylcholine mustard aziridinium, vincristine, aluminum, glutamate, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, and T3 deprivation. Aspects of this work are described, including mechanistic investigations in rat brain reaggregate cultures. In vitro exposure of xenobiotics through a tiered testing system (ranging from simple cell-based assays measuring cytotoxicol. parameters to more complex markers in organotypic cultures) may permit detection of central nervous system neurotoxicity in the contexts of both screening and mechanistics. The degree of simplicity, automaticity, and transportability of the tests requires consideration as will the possibility of endpoints for specific classes of chems., for example, cholinesterase for organophosphorus insecticides. Factors such as extrapolation from the central nervous system to the peripheral nervous system, metabolic activation, the blood-brain barrier, degree of neural cell activation, repair mechanisms, and developing vs. adult nervous systems are considered.

47

Mandys V, Turecek R, Gispen WH, Bar PR. ORGANOTYPIC CULTURES OF CHICK DORSAL ROOT GANGLIA IN A SEMI-SOLID MEDIUM: A MODEL FOR NEUROTOXICITY TESTING. Toxicol in Vitro 1994;8(1):81-90.

Organotypic cultures of dorsal root ganglia (DRGs) were isolated from 12-day-old chick embryos and cultured in a semi-solid medium for use as a model for toxicol. studies. The mean radial length of neuritic processes growing out from the ganglia, the daily rate of neurite elongation, and the area of neurite outgrowth were used as parameters to evaluate the toxic influence of three

drugs (cisplatin, taxol and chlorpromazine) that have neurotoxic side-effects through different modes of action on the peripheral nervous system. A significant, dose-dependent decrease of all parameters of neurite outgrowth was obsd. in cultures treated with different concns. of the chems. ested. The authors conclude that the culture system described here may be a useful in vitro method to test target organ toxicity in the peripheral nervous system.

48
Zachor D, Cherkes JK, Fay CT, Ocrant I. COCAINE DIFFERENTIALLY INHIBITS NEURONAL DIFFERENTIATION AND PROLIFERATION IN VITRO. Journal of Clinical Investigation 1994;93(3):1179-1185.

The outcome of in utero cocaine exposure is unclear. To determine if cocaine affects neuronal growth and differentiation, we used PC-12 cells, which have a mitogenic response to IGF-I and

differentiate into neurons on exposure to nerve growth factor. Differentiation was quantified as neurite extension after a 72-h exposure to 20 ng/ml nerve growth factor (dosage at 50% maximal effectiveness) and cocaine doses ranging from 0.01 to 10 mug/ml. The results were 49 : 2, 40 : 3, 23 : 2, and 12 : 2% differentiation with respective cocaine concentrations of 0, 0.01, 0.1, 1, and 10 mug/ml ($P < 0.0001$). Cocaine stability studies showed insignificant spontaneous hydrolysis under the conditions of this study. Cocaine did not affect cell viability or number, but had a relatively modest, statistically significant ($P < 0.0001$) inhibitory effect on IGF-I-stimulated thymidine incorporation. The dose-response curves for differentiation vs mitogenic response differed significantly ($P = 0.021$). Therefore, cocaine inhibition of these processes is probably mediated by different mechanisms, and not caused by generalized toxicity. To our knowledge, this is the first demonstration of cocaine effects on neuronal multiplication and differentiation in vitro. The results suggest in utero exposure may directly impair brain development.

49
Husain K. PHENYL VALERATE AND CHOLINE ESTER HYDROLASES IN THE PLATELETS OF HUMAN, HEN, RAT AND MOUSE. Human & Experimental Toxicology 1994;13(3):157-159.

The levels of phenyl valerate and choline ester hydrolases in the platelets of human and certain laboratory animals have been determined for comparison. The activities of total phenyl valerate hydrolase (PVase), paraoxon insensitive phenyl valerate hydrolase (PI-PVase), paraoxon and mipafox resistant esterase (PMRE) and propionylcholinesterase (PChE) were maximal in hen

followed by mouse, rat and human. Neurotoxicesterase (NTE) and acetylcholinesterase (AChE) activities were concentrated in the platelets of hens followed by humans, rats and mice in order. Maximum concentration of butyrylcholinesterase (BChE) was found in the platelets of hens followed by mice, humans and rats. It is concluded that the normal levels of enzyme activities in the platelets of humans and various species of animals may help to evaluate the exposure risk to neurotoxic organophosphorous compounds.

OCULAR TOXICITY

50

Cronin MT D, Basketter DA, York M. A QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP (QSAR)INVESTIGATION OF A DRAIZE EYE IRRITATION DATABASE. Toxicol in Vitro 1994;8(1):21-8.

National and international regulations require that the ocular safety of new chems. is established and demand that, unless the substance can be predicted to be harmful to the eye, this will be established by means of the Draize rabbit eye test. To date, no alternative in vitro methods are accepted by the regulatory authorities. Investigation of correlations between physicochem. properties of a chem. and its eye irritation potential has received scant attention. A 'gold std.' dataset of eye irritation tests collated by the European Center for Ecotoxicol. and Toxicol. of Chems. has been supplemented by other published data to form a consistent set of biol. activities for QSAR modeling. Using this dataset, the applicability of QSAR methods for modeling the eye irritation responses has been assessed. The possibilities, but also clear limitations, of a QSAR approach to the evaluation of eye irritation potential have been demonstrated. The dataset is the best available, but nevertheless presents several problems: the no. of irritants is small compared with the no. of non-irritants and the whole dataset is chem. heterogeneous, with the implication that multiple mechanisms of eye irritation may be involved; the data come from several labs.; the numerical Draize score is a pseudo-quant. parameter based on a summation of manipulated subjected scores. A molar adjusted eye irritation score proved to be at least partially useful in demonstrating and understanding the structure-activity relationships involved with such an endpoint. A more promising approach proved to be pattern recognition techniques using principal component anal. and relying on the overall classification of eye irritation potential. This has led to a qual. and, importantly, testable, relationship between eye irritation potential and physicochem. properties, particularly those describing hydrophobicity.

51

Rachui SR, Robertson WD, Duke MA, Paller BS, Ziets GA. PREDICTING THE OCULAR IRRITATION POTENTIAL OF COSMETICS AND PERSONAL CARE PRODUCTS USING TWO IN VITRO MODELS. In Vitro Toxicol 1994;7(1):45-52.

Forty-four materials (personal care products and cosmetics) were evaluated in Advanced Tissue Sciences' skin model ZK1200 and the bovine eye corneal opacity and permeability protocol to det. their in vitro ocular irritation potential. The ZK1200 assay was conducted using a protocol developed specifically for this model by the Procter & Gamble company. In vivo ocular irritation scores for these compds. ranged from nonirritating to severely irritating in both models. Cytotoxicity values, obtained via MTT redn., were used to rank-order the test materials in the ZK1200 tissue system as well as to classify and group them according

to their ocular irritation potential. Two endpoints, opacity change and permeability to fluorescein dye (0.4-0.5%), were examd. in the bovine eye model. These two endpoints were combined to arrive at a final in vitro score in the bovine system. The human skin model and protocol as well as the bovine eye protocol used in this research appear to be promising in vitro tools for

categorizing these test materials and offer a high degree of correlation with published in vivo results.

52
Lewis RW, McCall JC, Botham PA. USE OF AN IN VITRO TEST BATTERY AS A PRESCREEN IN THE ASSESSMENT OF OCULAR IRRITANCY. Toxicol in Vitro 1994;8(1):75-9.

The current OECD guideline for the assessment of eye irritation recommends, in initial considerations, the use of data from skin irritation tests as a prescreen to detect the most severely irritating materials, assuming that materials that are severely irritating to the skin are also significantly irritating to the eyes. However, anal. of data for 179 materials tested in this lab. for both dermal and ocular irritancy, revealed that, at most, only 36% of severe eye irritants were also severe skin irritants. This resulted in a significant no. of rabbits developing severe ocular effects that had not been predicted from the dermal responses. This study reports the results of an alternative approach for predicting severe eye irritants. The approach was a two-stage test battery in vitro: the first stage was a cytotoxicity assay utilizing the K562 cell line; the second was the isolated rabbit eye test. In contrast to the use of skin irritation tests, the in vitro battery was significantly more predictive (83% of severe eye irritants were detected). Although the incidence of false pos. responses in each of the assays precludes their routine use as a replacement from the in vivo

rabbit eye test they provide a powerful aid to reducing animal use and guiding in vivo studies to minimize the severity of effects. The need for an interlab. assessment to confirm and extend these findings is discussed.

53
Gautheron P, Duprat P, Hollander CF. INVESTIGATIONS OF THE MDCK PERMEABILITY ASSAY AS AN IN VITRO OF OCULAR IRRITANCY. In Vitro Toxicology 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 observed in parallel with the irritant potential of the test substances. The objective of the present study was to determine the usefulness of this test as a method for screening process intermediates, with a set of 42 reference chemicals. Surfactants were the most active substances with a ranking similar to in vivo ocular irritancy. Among the other chemicals tested, the most potent in vitro were classified as severely irritating in vivo, but the reverse was not always true. In addition, moderate and mild irritants appeared to be poorly differentiated in vitro. For surfactant and miscellaneous chemicals, 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, alcohols 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 observed between fluorescein permeability values and partition coefficients for a group of 13 alcohols 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 chemicals, at least with the methodology employed. Finally, hydrophobic properties of chemicals may play a direct role in this test system as in some other cell culture-based assays.

54
Chu I, Toft P. RECENT PROGRESS IN THE EYE IRRITATION TEST. Toxicology and Industrial Health 1993;9(6):1017-1025.

The rabbit eye irritation test based on the Draize method is required for the hazard assessment of chemicals and products that may come into contact with the eye. Due to the potential for the suffering of animals and subjectivity of the test, many modifications of the method have been made that involved a reduction in the number of animals and a refinement of techniques. Additionally, there has been significant development of in vitro alternatives. This paper reviews recent advances in

the in vivo test and in vitro alternatives, as well as regulatory requirements. While the refinement of in vivo protocols has resulted in a reduction in the number and discomfort on animals, the development of in vitro alternatives could lead to an eventual replacement of animal studies. In view of the inherent simplicity of many in vitro methods, some of which comprise cell cultures, further research into the relevance/mechanism of effects is required Batteries of in vitro tests, when properly validated, may be considered as replacements for animal testing.

ORGAN CULTURE

55

Abu-Musa A, Takahashi K, Okada S, Sakoda R, Kitao M. SERUM FROM PATIENTS WITH THREATENED ABORTION. EFFECT ON IN VITRO DEVELOPMENT OF MOUSE EMBRYOS. J Reprod Med 1994;39(1):10-2.

The effect of serum from patients with threatened abortion on in vitro development of two-cell mouse embryos was evaluated. Embryos were cultured for 72 hours in 7.5% serum supplement from patients with threatened abortion and from women with normal pregnancies and in Ham's F-10 medium with no serum supplement. Significantly fewer embryos developed to the blastocyst stage at 72 hours in the threatened abortion group as compared with the normal pregnancy group and Ham's F-10 ($P < .001$). These results suggest the presence of serum factors that are toxic to early embryonic development and thus may play a role in the pathogenesis of threatened abortion.

REGULATORY TOXICOLOGY

56

Haws LC, Jackson B, Turnbull D, Dressler W. TWO APPROACHES FOR ASSESSING HUMAN SAFETY OF DISPERSE BLUE 1. Regul Toxicol Pharmacol 1994;19(1):80-96.

Disperse Blue 1 is an anthraquinone dye used at low levels in semipermanent hair color formulations. Dietary administration of Disperse Blue 1 in a National Toxicol. Program (NTP) carcinogenesis bioassay produced transitional- and squamous-cell tumors, leiomyomas, and leiomyosarcomas of the urinary bladders of male and female F344/N rats. The occurrence of tumors in the urinary bladder of rats was assocd. with urothelial hyperplasia and the presence of urinary calculi. Despite the occurrence of urinary bladder calculi and other nonneoplastic changes, there was no evidence of urinary bladder carcinogenesis in B6C3F1 mice fed Disperse Blue in the diet for up to 2 yr. A study conducted in rats of the same strain by Burnett and Squire confirmed the occurrence of calculi and transitional-cell neoplasms in the rat bladder. However, no mesenchymal-cell tumors were detected at a

comparable dietary level. Further, Burnett and Squire found evidence of reversibility of the proliferative changes in the rat urinary bladder following cessation of treatment at 6 mo. Disperse Blue 1 has been tested in a variety of in vivo and in vitro genotoxicity assays and was neg. in vivo but produced a weak and mixed pattern of genotoxic responses in vitro which may be attributable to a constituent of the com. prepns. Evaluation of the available data for Disperse Blue 1 and comparison with the responses obsd. in the urinary bladders of rats administered other rodent bladder carcinogens considered to act through a secondary mechanism indicate that a threshold approach is appropriate for assessing risk. With this approach, an uncertainty factor of 1000 applied to the no-observed-adverse-effect level in the NTP bioassay yielded a safe exposure level of 45-56 mug/kg/day. In contrast, with a conventional quant. risk assessment approach, the exposure level corresponding to an upper limit on lifetime risk of 10-6 to 10-5 was 0.39 to 3.9 mug/kg/day, resp. The safe level of Disperse Blue 1 derived using the threshold approach is approx. 20 times greater than the max. av. daily applied dose of 2.7 mug/kg/day assocd. with its use in semipermanent hair color formulations, while the exposure assocd. with the 10-5 risk level using the linearized multistage model in the conventional approach was detd. to be 1.5 times greater. Because oral absorption is substantially more than dermal absorption, the actual margin of safety is most likely much greater than either of these comparisons suggests. The difference in the ests. derived using the two approaches demonstrates the importance of incorporating information on the mechanism of action into the risk assessment process.

REPRODUCTIVE TOXICITY

57
Perreault SD, Jeffay SC. STRATEGIES AND METHODS FOR EVALUATING THE FUNCTIONAL CAPACITY OF OOCYTES AND ZYGOTES IN VITRO. Methods Toxicol 1993;3B(Female Reproductive Toxicology):92-109.

Methods are described for the evaluation of oocyte and zygote function during oocyte maturation and fertilization. The utilization of these methods in support of toxicol. studies is discussed.

58
Polgar K, Yacono PW, Hill JA, Anderson DJ, Lee CY, Golan DE. USE OF THE TRANSLATIONAL MOBILITY OF A PLASMA MEMBRANE PROTEIN TO ASSESS FERTILIZATION OF MOUSE OOCYTES AND VIABILITY OF MOUSE ZYGOTES AND TWO-CELL EMBRYOS. Biol Reprod 1994;50(3):474-80.

The fluorescence photobleaching recovery (FPR) technique was used to measure the translational mobility of a glycoprotein

recognized by the monoclonal antibody (mAb) S75 in plasma membranes of mouse oocytes, zygotes, and two-cell embryos. Glycoprotein fractional mobility (f) was significantly decreased in membranes of unfertilized oocytes compared to zygotes or two-cell embryos (f values, 46 +/- 2 and 65 +/- 2%, respectively; $p < 0.0001$). Reduced apparent glycoprotein mobility was also observed in morphologically degenerated zygotes and two-cell embryos compared to viable zygotes and two-cell embryos (f values, 8 +/- 1 and 60 +/- 3%, respectively; $p < 0.0001$). These results indicate that the FPR technique can be used to assess oocyte fertilization and preimplantation embryonic viability. This method may be useful in the evaluation of embryonic viability following in vitro fertilization and in the detection of toxic effects of novel compounds on embryonic development.

SKIN/DERMAL TOXICITY

59

Koschier FJ, Roth RN, Stephens TJ, Spence ET, Duke MA. IN VITRO SKIN IRRITATION TESTING OF PETROLEUM-BASED COMPOUNDS IN RECONSTITUTED HUMAN SKIN MODELS. J Toxicol, Cutaneous Ocul Toxicol 1993;13(1):23-37.

Seven petroleum-based test materials were evaluated for skin irritation potential using full-thickness reconstituted human skin models (skin2 Model ZK1300 from Advanced Tissue Sciences, Inc., and Living Skin Equiv. (LSE) from Organogenesis, Inc.). Test materials were dosed undiluted in triplicate onto the epidermal side of the tissues that were incubated in polyethylene bags designed to minimize cross-contamination and loss of volatile constituents. The endpoints measured were thiazolyl blue (MTT) redn. and lactate dehydrogenase (LDH), prostaglandin E2 (PGE2), and interleukin-1 alpha (IL-1 alpha) release into spent media from treated and untreated tissues. Data from these tests were correlated with Draize primary dermal irritation index (PDII) scores using Cooper's criteria. Results showed that all endpoints measured approximated the skin irritation potential of the test materials, with the exception of IL-1 alpha in the skin2 Model ZK1300, in which significant levels of IL-1 alpha could not be detected. Further refinement and validation of this methodol. may provide industrial scientists with a practical method for evaluating volatile test materials in com. available tissue culture.

60

Osborne R, Perkins MA. AN APPROACH FOR DEVELOPMENT OF ALTERNATIVE TEST METHODS BASED ON MECHANISMS OF SKIN IRRITATION. Food Chem Toxicol 1994;32(2):133-42.

The authors' work is directed towards the development of cultured

human skin cells, together with endpoints that can be linked to in vivo mechanisms of skin irritation, as in vitro models for prediction of human skin irritation, and for study of mechanisms of contact irritant dermatitis. Three types of com. human skin cell cultures have been evaluated, epidermal keratinocytes and partially or fully cornified keratinocyte-dermal fibroblast co-cultures. Human epidermal keratinocyte cultures (Clonetics) were treated with product ingredients and formulations, and the extent of cell damage was assessed by incorporation of the vital dye neutral red. Cell damage correlated with human skin patch data for ingredient chems. with the exception of acids and alkalis, but did not correlate with skin irritation to surfactant-contg. product formulations. Cultures of human skin equiv. were evaluated as potential models for measurement of responses to test materials that could not be measured in the keratinocyte/neutral red assay. The authors developed a battery of in vitro endpoints to measure responses to prototype ingredients and formulations in human epidermal keratinocyte-dermal fibroblast co-cultures grown on a nylon mesh ('Skin2' from Advanced Tissue Sciences) or on a collagen gel ('Testskin' from Organogenesis). The endpoints measure cytotoxicity (neutral red and MTT vital dye staining, lactate dehydrogenase and N-acetyl glucosaminidase release, glucose utilization) and inflammatory mediator (prostaglandin E2) Release. Initial expts. indicate a promising correlation between responses of the Skin2 model to prototype surfactants and in vivo human skin irritation. The responses of Testskin cultures to acids and alkalis help to prove the concept that a topical application model can measure responses to these materials. These results suggest that human skin cell models can provide useful systems for preclin. skin irritation assessments, as alternatives to rabbits, for at least certain classes of test substances.

61
Rhoads LS, Cook JR, Patrone LM, Van Buskirk RG. A HUMAN EPIDERMAL MODEL CAN BE ASSAYED EMPLOYING A MULITPLE FLOURESCENT ENDPOINT ASSAY AND THE CYTOFLOUR 2300. J Toxicol, Cutaneous Ocul Toxicol 1993;12(2):87-108.

A human epidermal model (HEM) was developed that could be rapidly and automatically assayed in the CytoFluor 2300 (Millipore Corporation, Bedford, MA) spectrofluorometer using multiple site-and activity-specific fluorescent probes. The HEM was cultured on the optically translucent Millipore Millicell CM microporous membrane. Application of a variety of fluorescent dyes to this membrane without the HEM revealed negligible nonspecific dye assocn. The HEM was differentiated on a cross-linked collagen matrix and the latter was also found to retain less dye than the HEM. Feasibility-expts. using the site/activity-specific dyes calcein-AM (plasma membrane integrity

indicator), sodium fluorescein (epidermal permeability indicator), 5-chloromethylfluorescein diacetateacetoxymethyl ester (CMFDA-AM; intracellular glutathione level indicator), rhodamine 123 (mitochondrial activity indicator), neutral red (lysosomal integrity indicator), Fluo3-AM (intracellular calcium indicator), and a similar human epidermal model, the Organogenesis Test skin living skin equiv. (LSE), indicated that in vitro human epidermis might be amenable to automated anal. in the CytoFluor 2300. To det. if these fluorescent probes might reveal mechani sms underlying cytotoxicity, normal human epidermal keratinocyte (NHEK) monolayers were exposed to the single arm mustard, 2-chloroethylethylsulfide (CEES) and labeled with the aforementioned fluorescent probes. The data reveal that there is a dose- and time-dependent alteration in cellular activities due to mustard exposure, which, in turn, suggested a possible sequence of mustard toxicity. The fact that these HEMs can be analyzed automatically using the CytoFluor 2300 and that changes in specific physiol. parameters can be assessed using multiple fluorescent dyes suggests that this process might be a high throughput manner in which to screen further skin irritants.

62
Michel M, Germain L, Auger FA. ANCHORED SKIN EQUIVALENT CULTURED IN VITRO A NEW TOOL FOR PERCUTANEOUS ABSORPTION STUDIES. In Vitro Cellular & Developmental Biology Animal 1993; 29A(11):834-837.

No abstract.

63
Fullerton A, Broby-Johansen U, Agner T. SODIUM LAURYL SULPHATE PENETRATION IN AN IN VITRO MODEL USING HUMAN SKIN. Contact Dermatitis 1994;30(4):222-225.

Because of their ability to impair the skin barrier function, detergents constitute a major risk factor for the development of irritant contact dermatitis. Sodium lauryl sulphate (SLS) is a commonly used detergent for experimental studies within the area of irritant contact dermatitis. In the present study, penetration of S35-labelled 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 amount 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.

64
Kasparkova V, Hybasek P. EFFECT OF HERBICIDES ON HUMAN SKIN: PENETRATION OF HERBICIDES THROUGH THE EPIDERMIS "IN VITRO". Acta Universitatis Palackianae Olomucensis Facultatis Medicae 1992;133(0):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 Calderbank and Yuen was modified for quantitative determination of efficient components (paraquat, diquat) of both herbicides. Penetration of the chosen herbicides was different and surfactants contained in herbicides were found to play a role in penetration into the skin.

65
Eun HC, Jung SY. COMPARISON OF IRRITANT POTENTIAL OF SHAMPOOS USING CULTURED HUMAN EPIDERMAL KERATINOCYTES MODEL AND PATCH TEST REACTION MEASURED BY LASER DOPPLER FLOWMETRY. Contact Dermatitis 1994;30(3):168-71.

The keratinocyte culture model has previously been used as an in vitro method for testing skin irritating potential of common skin irritants. However, solubility limits its use for finished products. Shampoo is very soluble in water which should make it an ideal product category for the cell culture model. To determine the skin irritant potential of several commercial shampoos, we employed cultured human keratinocytes as an in vitro model. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide test (MTT) and lactic dehydrogenase release (LDH) test were used to document cell toxicity. 7 volunteers were patch tested and their reactions evaluated using laser Doppler flowmetry and compared with the in vitro data. MTT and LDH have a good negative correlation with each other. Patch test reaction, especially at high concentrations, correlates relatively well with the in vitro test, especially with shampoos of strong and weak irritancy. However, the rank order of the shampoos of moderate toxicity was not the same as in the in vitro data. This suggests that the cell culture technique cannot directly replace in vivo methods, and that data obtained by the cell culture method should be interpreted carefully.

TERATOGENECITY
66
Mahadevan MM, Weitzman GA, Hogan S, Breckinridge S, Miller MM. METHYLENE BLUE BUT NOT INDIGO CARMINE IS TOXIC TO HUMAN LUTEAL CELLS IN VITRO. Reprod Toxicol 1993;7(6):631-3.

Methylene blue (MB) is reported to be teratogenic when injected

intra-amniotically. Indigo carmine (IC) appears to be a safe alternative. To det. if MB has potential detrimental effects on ovarian tissue, the authors compared the effect of MB and IC on human granulosa luteal cell (GC) function in vitro. Human oocyte-cumulus complexes were obtained during in vitro fertilization cycles and one to three were placed in an organ culture dish. After insemination with sperm, oocytes were removed the day after retrieval and the attached GC were washed daily for 3 more days by changing 2 mL of culture medium. All the dishes were treated with human chorionic gonadotropin (hCG) for the next 24 h and progesterone (P) prodn. during this interval was taken as baseline. Test chems. were added with hCG for the next 48 h with daily media changes. The P prodn. during the last 24 h of chem. treatment was expressed as a percentage of the baseline. MB significantly reduced P prodn. whereas IC did not appear to have any effect. Moreover, under inverted microscopy more than 90% of the GC cells contained several small bluish intracellular granules when exposed to 0.01% MB but not 0.01% IC. These results indicate that MB may be taken up and processed by GC cells and inhibits P prodn. This finding adds to previous reports on the use of in vitro GC assay to identify potential reproductive toxicants.

67
Mihalikova K, Ujhazy E, Braxatorisova E, Kucera P. COMPARATIVE TERATOLOGICAL STUDY OF STOBODIN IN VIVO AND IN VITRO. Toxicol in Vitro 1993;7(6):803-7.

Stobadin (STO) is a prospective cardioprotective drug with antiarrhythmic and antihypoxic effects on the myocardium. Single i.v. injections of stobadin administered to rats on days 3, 6, 9 or 12 of gestation at doses of 2 and 6 mg/kg had no teratogenic effect. Slight fetal toxicity was manifested by decreased fetal wt. (day 3 of gestation, 6 mg/kg) and increased incidence of delayed ossification of the skull (day 12 of gestation, 6 mg/kg). In vitro studies were performed on chick embryos explanted at Hamburger and Hamilton (HH) stages 4-5 and cultivated in a medium with stobadin concn. ranging from 10-3 to 10-8 mol/L under std. conditions. Concns. of 10-3 and 10-4 mol/L were lethal. Embryos treated with concns. from 10-8 to 10-5 mol/L were comparable to those of the control group. The results of in vivo and in vitro tests showed that the antiarrhythmic agent stobadin at concns. up to the maximal i.v. therapeutic dose had no overt effects on different developmental stages of the rat embryo and early chick embryogenesis.

68
Guest I, Buttar HS, Smith S, Varma DR. EVALUATION OF THE RAT EMBRYO CULTURE SYSTEM AS A PREDICTIVE TEST FOR HUMAN TERATOGENS.

Can J Physiol Pharmacol 1994;72(1):57-62.

Ingestion of the anticonvulsant drug valproic acid and of the angiotensin converting enzyme inhibitor captopril during pregnancy has been assocd. with abnormal feed outcome in humans. In contrast, the use of the antiinflammatory drug ibuprofen and the antihistamine diphenhydramine has not been documented to be embryotoxic in humans. The authors evaluated the rat embryo culture system as a predictive model of teratogenesis, using these four drugs as test agents. Valproic acid, ibuprofen, and diphenhydramine were embryotoxic, inducing concn.-dependent decreases in growth and a significant increase in anomalies. Valproic acid caused an increase in neural tube defects, ibuprofen increased the incidence of abnormal maxillary processes, and diphenhydramine increased the no. of embryos with distorted body morphol. These abnormalities were induced at concns. of valproic acid and diphenhydramine that are used clin., but ibuprofen only induced toxicity at concns. greatly exceeding the therapeutic range. Captopril was not embryotoxic up to 5 mM, the highest concn. tested. These results suggest that the rat embryo culture system produces both false pos. and false neg. data on the teratogenic potential of drugs. Although such an in vitro assay may be suitable to det. the mechanism of teratogenesis, it is not a sensitive indicator of potential human

teratogens on its own. These data support the view that in vitro systems can only supplement clin. and epidemiol. observations in humans, possibly as a method to det. mechanisms of actions of teratogens.

TISSUE CULTURE
69
Becquet F, Goureau O, Soubrane G, Coscas G, Courtois Y, Hicks D. SUPEROXIDE INHIBITS PROLIFERATION AND PHAGOCYTIC INTERNALIZATION OF PHOTORECEPTOR OUTER SEGMENTS BY BOVINE RETINAL PIGMENT EPITHELIUM IN VITRO. Exp Cell Res 1994;212(2):374-82.

Experiments were performed to investigate the effect of free radical damage on two aspects of retinal pigmented epithelium (RPE) metabolism, namely, proliferation and phagocytosis. Bovine RPE cells were maintained in monolayer cultures, either as passaged (for proliferation and lysosomal activity assays) or primary cultures (for phagocytosis measurements). Free radicals (superoxide anions) were generated by a xanthine oxidase (XO)-hypoxanthine (HX) reaction. Total phagocytosis (binding plus ingestion of rod outer segments (ROS)) was quantitated by radioimmunoassay using a specific anti-opsin antibody and iodinated secondary antibody. In some cases, agents with known or possible protective influences against oxidative damage, i.e., superoxide dismutase (SOD), vitamin E, and basic fibroblast

growth factor (bFGF), were tested for their activity in this model system. RPE cell proliferation was inhibited in a HX-XO dose-dependent manner, in the absence of cell toxicity. Modifications of cell morphology were also noticed. Either simultaneous exposure of RPE cells to ROS membranes and HX-XO or pretreatment of ROS membranes with HX-XO prior to their addition to RPE monolayers led to a statistically significant 20-30% decrease in phagocytosis relative to control values. This decrease was essentially observed in the binding phase of phagocytosis, indicating damage to ROS surface molecules as the primary event. Addition of SOD or vitamin E prevented this loss of phagocytic activity, whereas bFGF had no effect. Superoxide radicals did not, however, affect phagocytosis when RPE cells were exposed to them alone, prior to incubation with ROS; nor did they alter a later stage in the phagocytic process, acid phosphatase activity. This tissue culture model represents a convenient system for analyzing free radical damage in different aspects of RPE-photoreceptor behavior and may be useful in studying this phenomenon in several retinal disorders.

TOXICITY (GENERAL)
70
Calleja MC, Persoone G, Geladi P. HUMAN ACUTE TOXICITY PREDICTION OF THE FIRST 50 MEIC CHEMICALS BY A BATTERY OF ECOTOXICOLOGICAL TESTS AND PHYSICOCHEMICAL PROPERITES. Food Chem Toxicol 1994;32(2):173-87.

Five acute bioassays consisting of three cyst-based tests (with Artemia salina, Streptocephalus proboscideus and Brachionus calyciflorus), the Daphnia magna test and the bacterial luminescence inhibition test (Photobacterium phosphoreum) are used to det. the acute toxicity of the 50 priority chems. of the Multicentre Evaluation of In vitro Cytotoxicity (MEIC) program. These tests and five physicochem. properties (n-octanol-water partition coeff., mol. wt., m.p., b.p. and d.) are evaluated either singly or in combination to predict human acute toxicity. Acute toxicity in humans is expressed both as oral LDs (HLD) and as lethal concns. (HLC) derived from clin. cases. A comparison has also been made between the individual tests and the conventional rodent tests, as well as between rodent tests and the batteries resulting from partial least squares (PLS), with regard to their predictive power for acute toxicity in humans. Results from univariate regression show that the predictive potential of bioassays (both ecotoxicol. and rodent tests) is generally superior to that of individual physicochem. properties for HLD. For HLC prediction, however, no consistent trend could be discerned that indicated whether bioassays are better estimators than physicochem. parameters. Generally, the batteries resulting from PLS regression seem to be more predictive than rodent tests or any of the individual tests. Prediction of HLD

appears to be dependent on the phylogeny of the test species: cructaceans, for example, appear to be more important components in the test battery than rotifers and bacteria. For HLC prediction, one anostracan and one cladoceran crustacean are considered to be important. When considering both ecotoxicol. tests and physicochem. properties, the battery based on the mol. wt. and the cladoceran crustacean predicts HLC substantially better than any other combination.

71
Fu L-J, Staples RE, Stahl R G JR. ASSESSING ACUTE TOXICITIES OF PRE- AND POST-TREATMENT INDUSTRIAL WASTEWATERS WITH HYDRA ATTENUATA: A COMPARATIVE STUDY OF ACUTE TOXICITY WITH THE FATHEAD MINNOW, PIMEPHALES PROMELAS. Environmental Toxicology and chemistry 1994;13(4): 563-569.

This study was undertaken to (a) determine wastewater treatment effectiveness using two freshwater organisms, (b) compare acute toxicity results from the two species exposed to the wastewaters, and (c) link acute and potential developmental toxicity of wastewaters in one organism. The acute toxicities of several pretreatment and post-treatment industrial wastewater samples were evaluated with adult Hydra attenuata (92- or 96-h exposure) and fathead minnows (96-h exposure). The acute LC50s agreed closely when results in Hydra attenuata were compared with those from fathead minnow tests. Acute LC50s ranged from 3 to > 100% of sample with hydra, and from 1.0 to > 100% of sample with fathead minnows. The results provided strong evidence of treatment effectiveness because toxicity decreased with progressive stages of treatment. Previously the Hydra Developmental Toxicity Assay (Hydra Assay) was used as a prescreen mainly for in vitro assessment of developmental toxicity with pure compounds and to prioritize toxicants according to selective toxicity to the developing embryo (A/D ratio). Recently we modified the assay for

testing natural waters and wastewaters; hence, some of the wastewater samples also were tested for their developmental toxicity. In this case, the relative selective toxicity (A/D ratio) of these wastewater samples ranged from 0.7 to 2. 1, indicating that no sample was uniquely toxic to the developing embryo, although acute toxicity was manifested. Overall, our results indicate the Hydra Assay functions appropriately in assessments of acute and developmental toxicity of industrial wastewaters and may be a simple and useful tool in a battery of tests for broader scale detection of environmental hazards.

VALIDATION TESTS
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Spielmann H, Kalweit S, Liebsch M, Wirnsberger T, Gerner I,

Bertram-Neis E, Krauser K, Kreiling R, Miltenburger HG, et al.
VALIDATION STUDY OF ALTERNATIVES TO THE DRAIZE EYE IRRITATION TEST IN GERMANY: CYTOTOXICITY TESTING AND HET-CAM TEST WITH 136 INDUSTRIAL CHEMICALS. Toxicol in Vitro 1993;7(4):505-10.

According to OECD guideline 405 revised in 1987 Draize eye tests need not be performed for severely irritating and corrosive chems. if results from 'well-validated alternative studies' are presented. In 1988 a validation study on alternatives to the Draize eye test was started in Germany to establish 'well-validated alternative methods' for this purpose. During database development, the last stage of the validation program, 136 chems. from the German chem. industry were classified in a blind trial with the 3T3 cell neutral red/kenacid blue cytotoxicity assay and the hen's egg chorioallantoic membrane (HET-CAM) test using fertile chicken eggs. The major goal of this stage of validation was to demonstrate the feasibility and limitations of the two alternative methods. Chems. were, therefore, selected as representatives of chem. structural groups as well as of physicochem. and toxicol. properties. In addn., some of the chems. were chosen because they were of interest to the cosmetic and detergent industries. Draize eye testing data in vivo were provided by industry. In contrast to data from a previous interlab. assessment trial, it was impossible to correlate cytotoxicity data to the EEC classification for in vivo eye irritation. However, seven of 10 severely irritating chems. (EEC labeling R-41) could be identified correctly in the HET-CAM assay, whereas test conditions of the study described here did not allow identification of irritating chems. (EEC labeling R-36). The HET-CAM test is, therefore, fulfilling the criteria of a 'well-validated alternative method' according to OECD guideline 405 and should be incorporated into eye irritation testing at the earliest possible stage to reduce effectively the suffering of rabbits in the Draize eye test. Although an 80% correct prediction of 'non-labeled' chems. in the HET-CAM test is encouraging, for safety assessment of non-irritant chems., for use as cosmetic formulations, for example, both government and industry will accept an in vitro assay only if its prediction of the absence of irritant properties is 100% correct.

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Kucera P, Cano E, Honegger P, Schilter B, Zijlstra JA, Schmid B.
VALIDATION OF WHOLE CHICK EMBRYO CULTURES, WHOLE RAT EMBRYO CULTURES AND AGGREGATING EMBRYONIC BRAIN CELL CULTURES [BY] USING SIX PAIRS OF CODED COMPOUNDS. Toxicol in Vitro 1994;7(6):785-98.

A comparative study was performed to assess the effects of 6 pairs of coded compds. on cultures of whole chick and rat embryos as well as aggregating brain cell cultures. Developed originally for basic studies in developmental

biol., these 3 culture systems have been adapted for the screening of chems. in the field of prenatal toxicol. Chick and rat embryos were cultured for 2 days during the early stages of organogenesis. Aggregating cell cultures were prepd. from early fetal rat telecephalon and grown for 14 days in a chem. defined medium. Concn.-response relationships were established by treating whole embryos in vitro for 2 days, and aggregating brain cell cultures for 9 days. After decoding the compds., the results showed that, in the 3 test systems, specific effects were induced at comparable concn. levels. Similar compd.-related malformations were found in both chick and rat whole embryo cultures. In aggregating brain cell cultures, neuron- and glia-specific effects could be distinguished. Based on the results obtained in the 3 in vitro systems, the following concn. ranges were detd. for the teratogenic/toxic potencies of the test compds. (in mol/L): <10-6: retinoids (Ro 13-6307, Ro 1-5488), 6-aminonicotinamide, ketoconazole; 10-6-10-3: 4-hydroxypyridine, sulfadiazine, sulfanilamide, caffeine, theophylline, metronidazole, methoxyacetic acid; >10-3: methoxyethanol. In general, the 3 in vitro test systems provided concordant and complementary data on the toxicity and teratogenicity of a given compd. These data were also comparable with those available from in vivo studies. It is concluded that such a test battery could contribute significantly to risk assessment and to the redn. of in vivo experimentation in reproductive toxicol.

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Cutz E, Speirs V, Yeger H, Newman C, Wang D, Perrin DG. CELL BIOLOGY OF PULMONARY NEUROEPITHELIAL BODIES-VALIDATION OF AN IN VITRO MODEL. I. EFFECTS OF HYPOXIA AND CALCIUM IONOPHORE ON SEROTONIN CONTENT AND EXOCYTOSIS OF DENSE CORE VESICLES. Anat Rec 1993;236(1):41-52.

Pulmonary neuroendocrine (NE) cells including the innervated clusters of NE cells-neuroepithelial bodies (NEB)-are difficult to study because of their small nos. and diffuse distribution within the airway mucosa of the lung. The authors have previously reported a method for isolation and culture of NE cells from rabbit fetal lung using a combination of mech. and enzymic dissocn. followed by gradient centrifugation. This method provides single cell suspension of mixed lung cells enriched in NE cells, particularly those originating from NEB. This study further validates this in vitro model by detailed morphol. characterization of cultured NEB cells using high resoln. light microscopy, transmission and SEM, HPLC for detection of 5-HT, and mol. (Northern blot) anal. of mRNA encoding for 5-HT synthesizing enzymes, tryptophan hydroxylase, and arom. L-amino decarboxylase. In addn. the effects of hypoxia on NEB cells in vitro were investigated to define the role of these cells as possible airway chemoreceptors. Exposure of NEB cultures

to hypoxia resulted in decreased intracellular content of 5-HT accompanied by increased exocytosis of dense core vesicles (DCV). The amt. of 5-HT release correlated with the degree of hypoxia, suggesting modulation by ambient pO2 levels. The role of Ca2+ ions in exocytosis of DCV and 5-HT release from NEB cells was tested in expts. with Ca2+ ionophore (A23187). Exposure of cultures to 5 mug/mL of ionophore resulted in up to 40% redn. in 5-HT content of NEB cultures as well as increased exocytosis of DCV. The overall findings are consistent with a view that NEB cells are chemosensory in nature and that Ca2+ signaling pathway is involved in stimulus-secretion coupling. Further refinements in cell sepn. and culture methodol. are required before more detailed investigation of NEB cell membrane properties, signal transduction mechanism, and intracellular signaling pathways can be carried out.

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Taniguchi Y, Suzuki K, Nakajima K, Nakajima M, Miwa Y, Yamada Y, Satoh M, Takeyoshi M, Akie Y, Moriyasu M, Yamamoto H, Katagiri M, Kazama A, Watanabe M. INTER-LABORATORY VALIDATION STUDY OF THE SKIN2 DERMAL MODEL ZK1100 AND MTT CYTOTOXICITY ASSAY KITS. Journal of Toxicological Sciences 1994;19(1):37-44.

An inter-laboratory validation study was conducted to evaluate the potential of 4 chemicals to cause irritation with 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-hexadecyl-pyridinium chloride monohydrate (CC), ethanol (EtOH), and dimethyl sulfoxide (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 laboratories 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 chemical 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 quantitative 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

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

Murine models for assessment of the contract sensitizing properties of chemicals rely on mouse ear swelling tests (Mest), which are not sensitive enough to detect weak sensitizers. The aim of the present study was to develop in mice and adjuvant-free Mest appropriate for in vivo detection of any type of sensitizer (weak to strong), and useful for in vitro assessment of contact sensitivity (CS). 3 haptens were tested: dinitrochlorobenzene (DCNB), para-phenylenediamine (pPD) and isoeugenol. We compared various protocols for induction of the CS reaction, differing by the site of induction, the number of applications and the concentrations of the 3 haptens. Comparison of the induction site for optimal Cs reaction showed that, in Balb/c mice, the back was a better site of induction than the abdomen. Detection of the sensitizing properties of weak sensitizers (pPD, isoeugenol) was possible using an adjuvant-free protocol, provided that the induction phase comprised hapten applications on 3 consecutive days on the backs of animals. For DNCB, one application was sufficient to obtain optimal CS reaction. For all 3 haptens, a secondary response in vitro was obtained using semi-purified lymph node T cells from animals sensitized 5 days before with the optimized Mest. These results demonstrate that the Mest could be a useful xperimental model for the study of all types of contact sensitizers.

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Underhill LA, Dabbah R, Grady LT, Rhodes CT. ALTERNATIVES TO ANIMAL TESTING IN THE USP-NF: PRESENT AND FUTURE. Drug Dev Ind Pharm 1994;20(2):165-216. (48 REFS)

A review of alternatives to animal testing in the U.S. pharmaceutical industry is presented, including physicochemical test, tissue culture, electronic and computer processing tests and databases, bioassays, combination testing, hormone potency assays, and general safety tests. Replacement of in vivo tests with in vitro and physicochemical tests has many advantages. Generally, animal tests are less sensitive than comparable in vitro or physicochemical tests. Also, alternatives to in vivo tests are usually less costly to run, and lend themselves well to multiple testing. Thirdly, animal responses may vary considerably due to genetic and handling variances, while in vitro and physicochemical tests tend to be more precise. Most importantly, animal welfare is a social concern, and seeking alternative tests

is a morally desirable action. A summary of the USP's efforts to seek alternative testing for biologicals and biologically derived products is presented.