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

A Bibliography with Abstracts

To Assist In:

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

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

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

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Suggestions and comments are welcome.

BRAIN/CNS
1
Weber SJ, Abbruscato TJ, Brownson EA, Lipkowski AW, Polt R, Misicka A, Haaseth RC, Bartosz H, Hruby VJ, Davis TP. ASSESSMENT OF AN IN VITRO BLOOD-BRAIN BARRIER MODEL USING SEVERAL [MET5]ENKEPHALIN OPIOID ANALOGS. J Pharmacol Exp Ther 1993;266(3):1649-55.

Confluent monolayers of primary and continuous passaged cultures of bovine brain microvessel endothelial cells (BMEC) have been suggested to model the blood-brain barrier (BBB). Increased lipophilicity has been previously suggested to increase BBB penetration. The intent of this study was to examine the effect that structural modifications of the [Met5]enkephalin analog DPDPE had on lipophilicity and passage across the BMEC. The BMEC consisted of a monolayer of confluent primary BMEC grown on polycarbonate (10 mum) filters. Permeability coeffs. were calcd. on the basis of the diffusion of peptides across the BMEC in a Side-Bi-Side diffusion chamber. Lipophilicity of the peptides examd. was detd. by using reversed-phase HPLC and calcg. the capacity factor (k). Diffusion across the BMEC (for all peptides examd.) was linear from 15 to 120 min; therefore, these time points were used to calc. permeability coeffs. Permeability coeffs. ranged from 14.34 to 92.00 cm/min (.times. 10-4), with [p-ClPhe4,4']biphalin being the highest. Anal. of variance coupled with the Newman-Keuls test showed greater passage of select peptide analogs across the BMEC, including [p-ClPhe4,4']biphalin, [p-ClPhe4]DPDPE and reduced DPDPE. Interestingly, upon passage across the confluent monolayer, reduced DPDPE was converted to cyclized DPDPE. Calcd. HPLC k ranged from 3.82 to 12.50. The most lipophilic peptide (highest) examd. was acetylated Phe0-DPDPE. Anal. of the regression line of permeability coeffs. plotted against k yielded a correlation coeff. of 0.745. The data provided in this study offer strong evidence that increasing peptide lipophilicity enhances passage across the BMEC. The greatest BMEC permeability coeffs., though not the greatest k, were obtained with peptides having a chlorohalogenation at the Phe4 residue, suggesting that factors other than lipophilicity may play a role in BMEC passage. Comparison of the permeability coeffs. obtained from the BMEC system with those obtained from in vivo BBB studies suggest that the BMEC system may be very useful in predicting peptide (analog) passage across the in vivo BBB.

2
Liu CH, Higgins RJ, Buster D, Sanborn JR, Wilson BW.
THE EFFECT OF ORGANOPHOSPHATES ON A CHICKEN BRAIN OR SEA URCHIN EGG KINESIN-DRIVEN MICROTUBULE MOTILITY ASSAY. Toxicol Lett 1993; 69(3):239-47.

The effect of neuropathic and non-neuropathic organophosphates (OPs) and acrylamide on an in vitro kinesin-driven microtubule (MT) motility assay was compared. The goal of the study was to determine whether this in vitro assay could confirm that a mechanism of action of neuropathic OPs was to impair kinesin activity and, therefore, possibly fast axonal anterograde transport (FAAT) in vivo. For the authors' study, kinesin from chicken brain (CK) and sea urchin egg (SUK) was initially purified. Western immunoblotting confirmed the close antigenic homol. between CK and SUK, using a mouse monoclonal sea urchin kinesin heavy chain-specific antibody (SUK 4). In the presence of microtubules (MTs) and MgATP, both CK- and SUK-driven MT movement was measured using a video-enhanced differential interference contrast microscope system with computer-assisted anal. Using this assay system, the authors then tested sep. the effect of two neuropathic OPs (diisopropylfluorophosphate (DFP) and Ph saligenin phosphate (PSP) and a non-neuropathic OP (paraoxon (PO)) each at a concentration of 10-2 M at 27.degree.C. Additionally, the authors tested acrylamide (10-2 M), since it is one of the best-characterized neurotoxins impairing FAAT in vivo. The authors' results demonstrated that none of these compounds significantly affected kinesin-driven MT motility in vitro compared to the std. controls. Further, this assay system was thus not able to discriminate between the neuropathic and non-neuropathic effect of these OPs.

3
Funk KA, Liu C-H, Wilson BW, Higgins RJ. AVIAN EMBRYONIC BRAIN REAGGREGATE CULTURE SYSTEM: I. CHARACTERIZATION FOR ORGANOPHOSPHORUS COMPOUND TOXICITY STUDIES. Toxicol Appl Pharm 1994;124(1):149-158.

An avian reaggregate culture system was characterized biochemically and morphologically for use in acute and chronic organophosphorus compound (OP) toxicity studies. Ten-day-old chick embryo brains were dissociated, reaggregated, and maintained in a chemically defined, serum- and antibiotic-free media. Acetylcholinesterase (ACHE), neuropathy target esterase

(NTE), and 2',3-cyclic nucleotide 3'-phosphodiesterase (CNP) were examined due to inhibition of these enzymes as a result of acute OP toxicity (ACHE) or delayed toxicity (NTE, CNP). The selected enzymes also indicate reaggregate neuronal (ACHE, possibly NTE), oligodendroglial (CNP), and astrocytic (glutamine synthetase (GS)) activities. Enzyme activities were compared to those in age-matched chick embryo and hatched chick brains. Reaggregate ACHE specific activity was similar to or higher than that of chick embryo or hatched chick. Reaggregate NTE specific activity was initially similar to that of 10-day-old chick embryo, and then increased but subsequently averaged 7.8 nmol/min/mg protein. In chick brain, NTE peaked at hatching and averaged 28 nmol/min/mg protein thereafter. Reaggregate CNP specific activity ranged from 103 to 426 nmol/min/mg protein, whereas activity gradually increased in chick embryo brain to an average of 140 nmol/min/mg protein posthatching. The mean GS activity ranged from 0.15 (Culture Day 4) to 1.09 nmol/min/mg protein (Culture Day 62). Mean protein values per flask ranged from 2.47 to 7.58 mg. Ultrastructurally, myelination was detected at Culture Day 7 and synapses at Day 6. The biochemical and ultrastructural features demonstrate that this reaggregate culture is a practical and sensitive in vitro system for studying both the acute and the long-term neurotoxicological effects of organophosphorus compounds.

CANCER

4

Shaw GL, Gazdar AF, Phelps R, Linnoila RI, Ihde DC, Johnson BE, Oie HK, Pass HI, Steinberg SM, Ghosh BC, et al. INDIVIDUALIZED CHEMOTHERAPY FOR PATIENTS WITH NON-SMALL CELL LUNG CANCER DETERMINED BY PROSPECTIVE IDENTIFICATION OF NEUROENDOCRINE MARKERS AND IN VITRO DRUG SENSITIVITY TESTING. Cancer Res 1993; 53(21):5181-7.

We attempted to prospectively select individualized chemotherapy for 165 non-small cell lung cancer patients based on in vitro analysis of neuroendocrine (NE) markers and drug sensitivity testing (DST) using fresh tumor. The chemotherapy used for small cell lung cancer (SCLC) was selected when NE marker expression determined by L-dopa decarboxylase assay was documented. Selection of chemotherapy for other patients was guided by DST results using a modified dye exclusion assay when available; otherwise etoposide and

cisplatin was administered. A total of 112 of 165 (68%) specimens were assayed for L-dopa decarboxylase and 36 patients (22%) had DST. In vitro data directed management for 27 of 96 (28%) patients given chemotherapy: 6 with NE markers were treated with the SCLC regimen; and 21 (58% of those with DST) received their DST-selected chemotherapy regimen. There were no significant differences in response rate among all 3 treatment arms (P = 0.076). However, response to chemotherapy for the patients treated prospectively with a SCLC regimen was 3 of 6 (50%), marginally better than patients given their DST-selected chemotherapy regimen (2 of 21; 9%; P = 0.056) or those treated with etoposide and cisplatin (10 of 69; 14%; P = 0.061). When patients whose NE markers were identified retrospectively are included, 4 of 9 (44%) responded to administered chemotherapy, compared to 7 of 55 (13%) with no NE markers present (P = 0.04). There were no differences in survival among the three treatment groups. Cisplatin and etoposide comprised the most active regimen in vitro for tumors from 16 of 36 (44%) patients, potentially limiting the benefit of DST since this is often the empiric therapy for non-SCLC. Furthermore, the correlation between in vitro and clinical response is nonsignificant for all drugs tested, highlighting the overall relative resistance of non-SCLC tumors to currently available chemotherapy.

5
Kobayashi S, Okada S, Yoshida H, Hasumi T, Sato N, Inaba H, Nakada T, Fujimura S. A CONVENIENT AND INEXPENSIVE CHEMO-RADIOSENSITIVITY ASSAY FOR LUNG CANCER CELLS USING TERASAKI'S MICROPLATE. Tohoku J Exp Med 1993;171(1):65-75.

We devised a simple in vitro sensitivity test for lung cancer cells using Terasaki's microplate. We used the test to screen for sensitivity to various carcinostatic drugs and radiation, and to determine the optimum method of administration. This assay has been used in routine clinical examinations because about 40% of non-small cell carcinoma and 80% of small cell carcinoma of the lung can be subcultured. We describe here our patients who underwent treatment, various sensitivity tests and the preparation of an optimal course of treatment based upon the results of the sensitivity tests. Cells were placed in primary culture as previously described for short-term selective culture, and 2nd-3rd generation subcultured cells were

transferred to individual wells of Terasaki's microplates for various sensitivity tests. After culture for 10 days, the effect was evaluated using 0.1% iodonitrotetrazolium (INT). This test permits a variety of sensitivity tests and various studies of clinical models of intensive treatment to be performed conveniently and reproducibly, because subcultured cancer cells are used. Another advantage is that these cells can be applied to basic investigations, including the preparation of monoclonal antibodies and chromosomes, DNA ploidy and oncogene studies.

CARCINOGENESIS
6
Gold LS, Slone TH, Stern BR, Bernstein L. COMPARISON OF TARGET ORGANS OF CARCINOGENICITY FOR MUTAGENIC AND NON-MUTAGENIC CHEMICALS. Mutat Res 1993; 286(1):75-100. (35 REFS)

The distribution of tumors induced by 351 rodent mutagenic and nonmutagenic carcinogens was studied. The mutagenicity of the chemicals was determined by the results of Salmonella assays reported by the National Institute of Environmental Health Sciences Experimental Carcinogenesis and Mutagenesis Branch and the Environmental Protection Agency Gene Tox Program and carcinogenicity was determined from the Carcinogenic Potency Database. The number of mutagens demonstrating carcinogenicity was higher than the number of nonmutagens and multiple organ tumors were induced more frequently by mutagens than nonmutagens. The group of carcinogens with the strongest evidence of carcinogenicity contained a higher proportion of mutagens compared to those that did not demonstrate such dramatic carcinogenic effects. A table was presented listing 32 biological tissues and organs and the tested chemicals that induced tumors at these sites in either rats or mice. The liver was the most common target site for tumors for mutagens as well as nonmutagens in both rodent species. Over 80% of the tested chemicals were positive in at least one of eight target sites including: liver, lung, mammary gland, stomach, vascular system, kidney, hematopoietic system, and the urinary bladder. It was more common for chemically induced tumors to be found at more than one site than at a single site. A comparison was presented between the current results and those of another study examining the relationships between mutagenicity, carcinogenicity, and chemical structure and activity.

7
Ashby J, Brusick D, Myhr BC, Jones NJ, Parry JM, Nesnow S, Paton D, Tinwell H, Rosenkranz HS, Curti S, Gilman D, Callander RD. CORRELATION OF CARCINOGENIC POTENCY WITH MOUSE-SKIN 32P-POSTLABELING AND MUTA-(R)MOUSE LAC Z(-) MUTATION DATA FOR DMBA AND ITS K-REGION SULPHUR ISOSTERE: COMPARISON WITH ACTIVITIES OBSERVED IN STANDARD GENOTOXICITY ASSAYS. Mutat Res 1993;292(1):25-40. (52 REFS)

The genotoxic effects of 7,12-dimethylbenz(a)anthracene (DMBA) and its 4,5-sulfur analog,6,11-dimethylbenzo(b)naphtho- (2,3-d)thiophene(SDMBA)were analyzed and compared using different in-vitro and in-vivo assays. The genotoxicities of these compounds were tested using the Salmonella-typhimurium assay, a cell transformation assay using the mouse embryo fibroblast cell line C3H10T1/2, an in-vivo micronucleus assay in CBA-mice, and an in-vivo mouse skin mutation assay in CD(2)-LacZ80/HazfBR-mice mice. Both DMBA and SDMBA showed similar mutagenic effects in the Salmonella assay while only DMBA gave a strong positive response in the cell transformation assay. DMBA gave a positive response and SDMBA an essentially negative response in the micronucleus assay. SDMBA was mutagenic following topical application to mouse skin, however, it was not as potent as DMBA in this assay. Both compounds induced seven distinct major adducts which were identified in DNA adduct profiles obtained by phosphorus-32 labeling studies. DMBA induced adducts at a higher rate than did SDMBA. SDMBA was found to be half as soluble as DMBA in wet octanol. A Multicase structural analysis indicated that SDMBA was nonmutagenic to Salmonella and noncarcinogenic to rodents following oral administration, but carcinogenic upon topical application. Analysis of the molecular geometry of SDMBA indicated that it had a structure that was nonplanar and less distorted than DMBA. The data confirm the importance of conducting predictive genotoxicity assays in systems close to those in which carcinogenicity is to be assessed.

8
Benigni R, Andreoli C. RODENT CARCINOGENICITY AND TOXICITY, IN VITRO MUTAGENICITY, AND THEIR PHYSICAL CHEMICAL DETERMINANTS. Mutat Res 1993;297(3):281-292.

In this paper, we considered rodent carcinogenicity and

toxicity, and four in vitro mutagenicity systems, and we made a global comparison between their different response profiles to a common set of 297 chemicals. This analysis is complemented with a study of the physical chemical properties of active and inactive compounds in the different systems. A clearcut separation between the different classes of toxicological end-points (carcinogenicity, in vivo toxicity, in vitro carcinogenicity) was evident. The observed lack of association between carcinogenicity and toxicity supports the validity of the rodent bioassays; this is contrary to the position that the positive results obtained are due mainly to the use of excessive doses that exert cytotoxic effects. We found substantial consistency in the responses of the in vivo toxicity systems (maximum tolerated dose and LD50), but we also found that remarkable differences exist between the in vitro mutagenicity assay systems. The study of the structure-activity relationships showed that: (a) the hydrophobic-electronic properties of the chemicals influence rodent carcinogenicity, with the tendency of carcinogens to be more electrophilic and more hydrophobic than non-carcinogens; (b) steric effects are implied in in vitro mutagenicity, bulkier molecules being less mutagenic than smaller molecules; (c) no clear association between in vivo toxicity and physical chemical properties was apparent. The differences between carcinogenicity and in vitro mutagenicity may hypothetically be related to their different experimental procedures. The relatively short treatment of in vitro mutagenicity requires that chemicals penetrate easily into the cells, and are well dissolved into the aqueous medium, size and hydrophilicity thus being critical for the action of the chemicals. The size of the molecules is not critical in the long-term rodent carcinogenicity experiments, where other factors, like bioaccumulation (hydrophobicity) and electronic reactivity, become essential.

9
James N, Molloy C, Soames A, French N, Roberts R. AN IN VITRO MODEL SYSTEM TO INVESTIGATE HEPATOCYTE GROWTH REGULATION DURING RODENT NON-GENOTOXIC HEPATOCARCINOGENESIS. Meeting of the British Toxicology Society and the British Society of Toxicological Pathologists, Canterbury, England, UK, April 5-7, 1993. Human & Experimental Toxicology 1993; 12(6): 558.

No abstract.

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

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

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

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

substrata of type IV collagen mixed with laminin, basal medium with complex lipids and low calcium, specific growth factors (most potently, insulin-like growth factor II and granulocyte-macrophage colony-stimulating

factor), and co-cultures of embryonic, liver-specific stroma, strongly suggesting paracrine signaling between hepatic and hemopoietic precursor cells. The growing OCAP cultures proved to be uniformly expressing oval cell markers but were nevertheless a mixture of hepatic and hemopoietic precursor cells. To separate the hepatic and hemopoietic subpopulations of OCAP cells, we surveyed known antibodies and found ones that uniquely identify either hepatic or hemopoietic cells. Several of these antibodies were used in panning procedures and fluorescence-activated cell sorting to eliminate contaminant cell populations, particularly hemopoietic and endothelial cells. Using specific flow

cytometric parameters, three cellular subpopulations could be isolated separately that were identified by immunochemistry and molecular hybridization assays as probable: (i) committed progenitors to hepatocytes; (ii) committed progenitors to bile ducts; or (iii) a mixed population of hemopoietic cells that contained a small percentage of hepatic blasts that are possibly pluripotent. The hepatic precursor cells have been characterized using immunochemistry, flow cytometry, and molecular hybridization assays. The hepatic blasts are small (7-10 microns) cells with high nuclear to cytoplasmic ratios and with minimal complexity of the cytoplasm. Cultures of the committed progenitors were found to differentiate into cells with recognizable parenchymal cell fates. We discuss our studies in the context of our model of the liver as stem cell and lineage system and suggest that a slow, unidirectional, terminal differentiation process, paralleling more rapid ones in the skin or gut, occurs at all times in the liver and is thought to vary primarily in kinetics during quiescent versus regenerative states.

12
Cao J, Leibold E, Beisker W, Schranner T, Nuesse M, Schwarz LR. FLOW CYTOMETRIC ANALYSIS OF IN VITRO MICRONUCLEUS INDUCTION IN HEPATOCYTES TREATED WITH CARCINOGENS. Toxicology In Vitro 1993; 7(4):447-451.

The micronucleus test is frequently used in established cell lines to detect genotoxic chemicals. In contrast, there is only very limited experience concerning the

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

CARDIOTOXICITY

13

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

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

CELL CULTURE

14

Fang VS, Ho LT. A PRIMARY CELL-CULTURE SYSTEM FOR PHYSIOLOGICAL STUDIES OF ADRENOCORTICAL FUNCTION. Chin J Physiol (Taipei 1993);36(2):125-31.

The authors used guinea-pig adrenal tissue to develop a primary culture, enriched with zona fasciculata (ZF) cells. In a continuous culture .ltoreq.2 wk, the cells maintained the characteristic of glucocortical function by producing cortisol as the final steroidogenic product and secreting it into culture medium. When culture medium, which was replaced at 24-h intervals, was assayed for cortisol, the basal steroidogenic function peaked on day 5 and then declined. In response to 24-h treatment with the bioactive adrenocorticotropic hormones (ACTH) on day 4, prodn. of cortisol was stimulated and prolonged, and also cells were morphol. hypertrophic. This in vitro system provides a convenient lab. method which can be used for studying adrenocortical function under a possibly physiol. condition.

15

Cascorbi I, Bittrich H, Ricklinkat J, Voss W, Seyfarth A, Foret M. EFFECTS OF A HETEROGENOUS SET OF XENOBIOTICS ON GROWTH AND PLASMA MEMBRANES OF MAMMALIAN AND FUNGAL CELL CULTURES. Ecotoxicol Environ Saf 1993;26(1):113-26.

A comparison of the toxicity of 45 selected, heterogenous substances on two test organisms of different taxonomic levels, the yeast Saccharomyces cerevisiae and Chinese hamster ovary (CHO) cells, was made. In addn., effects on the yeast plasma membrane-integrated H+-ATPase and on the CHO adenosine uptake system were investigated. For all test systems, log EC50 values highly correlated with EC20 values. Good correlations were obtained between CHO proliferation rate and yeast growth rate (r = 0.80). However, CHO cells were about four times more sensitive than yeast. A good accordance was also found between effects on yeast cell growth and on the H+-ATPase, indicating a plasma membrane impairment as a major cause of cytotoxicity. These findings were supported by correlations of log EC20 values with the log Pow as a measure for lipophilicity. Although the test systems demonstrated different dependencies, the main trend reflected an increasing toxicity with increasing

lipophilicity. Comparisons with data from in vivo test systems suggest that these in vitro test systems could be implemented for initial estn. of basic toxicity and the detection of outliers thereby reducing the no. of tests with higher animals.

16
Genbacev O, White TEK, Gavin CE, Miller RK. HUMAN TROPHOBLAST CULTURES: MODELS FOR IMPLANTATION AND PERI-IMPLANTATION TOXICOLOGY. Reprod Toxicol 1993;7(Suppl 1):75-94. (113 REFS)

Experimental techniques for investigating implantation toxicity were discussed. Toxic agents can interfere with various phases of the implantation process. Up to 50% of the unexplained losses occurring during pregnancy have been attributed to disruption of preimplantation or implantation events by toxicants. In-vitro models for investigating implantation toxicology were discussed. Studies of the mechanisms underlying implantation toxicity are of necessity limited to using in-vitro test systems. In-vitro models of human implantation processes can be classified according to which phase they are designed to study. Most test systems have been developed and applied to investigating invasion of the uterine decidua and myometrium by trophoblasts. Such systems have used syncytiotrophoblasts, choriocarcinoma cells, and cytotrophoblasts. Matrix degrading proteolytic activity, invasion of basement membranes, and expression of receptors for extracellular matrix components have been used as endpoints. Other less widely used in-vitro test systems have assessed the effects of toxicants on blastocyst penetration of the endometrium and cytotrophoblast cell migration in fibronectin, laminin, and collagen matrices. In-vitro studies of xenobiotics that have been shown to alter human placental function were summarized. A large number of studies have investigated the effects of cadmium. These have shown that cadmium has the potential for interfering with implantational processes by exerting effects on trophoblasts and the uterine endometrium.

17
Soto AM, Lin T-M, Justicia H, Silvia RM, Sonnenschein C. AN "IN CULTURE" BIOASSAY TO ASSESS THE ESTROGENICITY OF XENOBIOTICS (E-SCREEN). Chemically-Induced Alterations in Sexual and Functional

Development: The Wildlife/Human Connection, T. Colborn and C. Clement, Editors; Advances in Modern Environmental Toxicology, Vol. XXI, Princeton Scientific Publishing Co., Inc., Prince, 1992.

An in-vitro bioassay for detecting estrogenic compounds was developed. The bioassay, known as the E-screen, was based on the premises that a human serum borne molecule specifically inhibits the proliferation of MCF7 cells, a human breast cancer estrogen cell line, and estrogenic xenobiotics induce cell proliferation by abolishing this inhibitory effect. Cloned MCF7 cells were cultured in medium containing 10% heat inactivated human serum rendered estrogenless by treatment with charcoal stripped dextran. The test chemical was added to the cultures and incubated with the cells in the presence or absence of 10(-13) to 10(-9) molar estradiol-17beta for 6 days. The number of cells were counted after 6 days from which the extent of proliferation was determined. The proliferative effect (PE), relative proliferative potency (RPP), and relative PE (RPE) of the test chemical were determined from the data. The PE was defined as the ratio between the highest cell yield obtained with the test chemical and the yield in the estradiol free culture. The RPP was defined as the ratio of the estradiol concentration needed to obtain a maximum cell yield to the minimum dose of the test compound required to produce a similar effect. The RPE was defined as the ratio between the maximum cell yield obtained with the test compound and estradiol. The assay was tested with 13 insecticides, phytoestrogens, and phytohormones, six natural and synthetic estrogens, and 16 alkyl or aromatic phenols. The authors conclude the E-screen is useful for screening biological materials for estrogenic substances before they are released into the environment.

18
Lee L EJ, Clemons JH, Bechtel DG, Caldwell SJ, Han K-B, Pasitschniak-Arts M, Mosser DD, Bols NC. DEVELOPMENT AND CHARACTERIZATION OF A RAINBOW TROUT LIVER CELL LINE EXPRESSING CYTOCHROME P450-DEPENDENT MONOOXYGENASE ACTIVITY. Cell Biol Toxicol 1993;9(3):279-294.

A cell line, RTL-W1, has been developed from the normal liver of an adult rainbow trout by proteolytic dissociation of liver fragments. RTL-W1 can be grown routinely in the basal medium, L-15, supplemented with 5% fetal bovine serum. In this medium, the cells have

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

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

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

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

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

No abstract.

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

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

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

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 muM) 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 in grained 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. A third focus should be the concept of Pb tolerance, particularly in astroglia, which adapt to and tolerate the presence of intracellular lead. Given the slow turnover of Pb in the brain, mechanisms for tolerance are of considerable importance. Until now, little research has been carried out on long-term exposure to Pb in culture. This issue, above all, requires attention in the intensification phase.

23
Warenius HM, Britten RA, Browning PG, Morton IE, Peacock JH. IDENTIFICATION OF HUMAN IN VITRO CELL LINES WITH GREATER INTRINSIC CELLULAR RADIOSENSITIVITY TO 62.5 MEV (P-->BE+) NEUTRONS THAN 4 MEV PHOTONS. Int J Radiat Oncol Biol Phys 1994; 28(4):913-20.

To identify human in vitro cell lines with a high relative cellular sensitivity to fast neutrons as compared to photons and to examine their relationship to intrinsic photon radiosensitivity and cellular proliferation kinetics. METHODS AND MATERIALS: The

clonogenic cell survival following exposure to low LET, 4 MeV photons or, high LET, 62.5 MeV (p-->Be+) fast neutrons and the cell kinetic parameters of 30 human in vitro cell lines, covering a wide range of histologies, were analyzed alone and with previously published data of Fertil and Malaise. The relative survival at 1.6 Gy of neutrons (SF1.6) compared to 2 Gy of photons (SF2) (the doses per fractions used in the Clatterbridge fast neutron studies) and the cell kinetic parameters of the 30 cell lines were also compared. The relative lethality of 62.5 MeV fast neutrons was assessed by comparing the ratio alpha neutrons/alpha photons to alpha photons or SF1.6 neutrons/SF2 photons to SF2 photons. Cellular proliferation kinetics were measured by flow cytometry following BrdU incorporation and the relationship of cellular proliferation to relative neutron lethality was measured by comparing the alpha neutron/alpha photon ratio to the labelling index (LI), potential doubling (Tpot) and ploidy. RESULTS: The majority of cell survival curves obtained following exposure to 62.5 MeV fast neutrons were curvilinear with beta values of similar order to those obtained with low LET 4 MeV photons. Comparison of alpha values for neutrons and photons revealed a relatively neutron sensitive subset of 9 out of 30 in vitro cell lines. This subset was not, however, distinguishable when 1.6 Gy of neutrons was compared to 2 Gy of photons. There

was no correlation between cell survival with neutrons or photons and the cell kinetic parameters Tpot or LI or with DNA ploidy. CONCLUSIONS: The use of in vitro

assays of neutron and photon radiosensitivity irrespective of cell kinetic parameters allows identification of neutron sensitive cell populations when the ratio of the alpha values for neutrons and photons is compared to the reciprocal of the alpha photon value. This relationship is not apparent when fractions of 2 Gy of photons are compared to 1.6 Gy of neutrons. Whether or not this identification can be borne out in fractionated regimes in the clinic remains to be proved.

24
Keilhoff G, Wolf G. COMPARISON OF DOUBLE FLUORESCENCE STAINING AND LDH-TEST FOR MONITORING CELL VIABILITY IN VITRO. Neuroreport 1993; 5(2):129-32.

To examine glutamate-mediated toxic effects in dispersed cultures of the rat cerebral cortex we compared the utility of parameters of cell viability in parallel. A 1 h exposure to glutamate and glutamate analogues (kainate, quinolinate, NMDA) was found to produce typical morphological changes in matured cell cultures. The double-labelling fluorescence technique (fluorescein diacetate/propidium iodide) reflected the degeneration process vividly. A significant increase in LDH-activity released into the medium was noted only when the cells were stressed but still living. When the phase of progressive cell death was running, LDH activity in the medium decreased markedly. Obviously, increasing LDH release in the culture medium must be considered rather to be an indicator for a slowly evolving degenerative process than for cell death.

25
Ellard S, Parry JM. A COMPARATIVE STUDY OF THE USE OF PRIMARY CHINESE HAMSTER LIVER CULTURES AND GENETICALLY ENGINEERED IMMORTAL V79 CHINESE HAMSTER CELL LINES EXPRESSING RAT LIVER CYP1A1, 1A2 AND 2B1 CDNAS IN MICRONUCLEUS ASSAYS. Toxicology 1993;82(1-3):131-149.

Liver microsome preparations (S9 mix) have been extensively used for in vitro genotoxicity studies to provide the capacity for the activation of indirect genotoxins. However, the use of S9 preparations with mammalian cell cultures has raised considerable

toxicity problems which limit their use to exposure times which are only a small fraction of the cell cycle. In addition, false negative results may be obtained if reactive metabolites are unable to penetrate the cell membrane or have short half-lives. The generation and detection of a promutagen within a single cell would therefore be advantageous. To this end, we have studied the bioactivation of a panel of promutagens (benzo(alpha)pyrene, cyclophosphamide, 2-aminoanthracene and sterigmatocystin)in low passage Chinese hamster fibroblasts of hepatic origin (LiC2 cells) and in a series of V79 Chinese hamster cell lines genetically engineered to express rat liver cytochrome P450 cDNAs. These include strains XEM2 (expresses CYP1A1), SD1 (CYP2B1) and strains XEMd-MZ and ZEMd-NH which express CYP1A2. The end point selected for study was the induction of micronuclei. The protocol incorporated a cytochalasin B-induced cytokinesis block and the enumeration of micronuclei in the resulting binucleate cells which have undergone one nuclear division following the induction of chromosome damage. Micronuclei containing whole chromosomes and chromosome fragments were distinguished by the use of CREST antibody specific for kinetochore protein as a measure for the presence of centromeres. Micronuclei were induced by the test agents in low passage liver fibroblasts and in immortal V79 cultures only in the presence of Aroclor-induced S9 preparations. The data obtained from micronucleus assays of the genetically engineered V79 cell lines demonstrated the utility of each strain for the optimal detection and quantification of the activity of the individual test compounds. Kinetochore antibody demonstrated differences in the kinetics of induction of micronuclei containing chromosome fragments and whole chromosomes with chemicals such as benzo(alpha)pyrene. As part of this cytogenetic study, we also conducted karyotypic analyses and spindle fidelity assays of the V79 cell lines to investigate the presence of chromosomal instabilities which may arise as a consequence of the genetic engineering procedure. Such studies represent an important quality control step in the validation of the suitability of each cell line prior to their use in genotoxicity studies.

26
Kasamaki A, Urasawa S. THE EFFECT OF FOOD CHEMICALS ON CELL AGING OF HUMAN DIPLOID CELLS IN IN VITRO CULTURE. J Toxicol Sciences 1993;18(3):143-153.

The potency of food chemicals to induce cell aging was evaluated in human diploid fibroblast cells HAIN-55 having a finite replicative potential by using in vitro aging markers, i.e., decreases of maximum proliferative potential (lifespan) of cells, saturation density in monolayer culture (SD), plating efficiency (PE) and mitotic index (MI), and an increase of cells with polyploid karyotypes. By treatment twice with low concentration of genotoxic chemicals aflatoxin B1, allylisothiocyanate or trans-cinnamaldehyde (severe clastogenic flavoring agent; Kasamaki et al., 1982), lifespan (expressed by the number of cumulative cell population doubling (CPD)) of the treated cells was reduced by 8-12 CPDs accompanied by change of the other aging markers. By successive treatment (29 of 25 times) with non-genotoxic chemical aspartame (N-L-aspartyl-L-phenylalanine) or L-canavanine (structural analogs of L-arginine), lifespan of the treated cells was also slightly shortened (by 2-6 CPDs) compared with the untreated control cells. In the process of cell aging, Mitochondrial activity (MTT activity) decreased almost in parallel with the decrease of SD and MI. On the basis of these results, a variety of genotoxic and non-genotoxic chemicals were examined by using MTT activity as the aging marker for their effects on the aging of HAIN-55 cells and bovine artery endothelial cells which also had a finite replicative potential. The results showed that seven genotoxic and nine non-genotoxic chemicals promoted cell aging.

27
Kremers P, Roelandt L, Todaro A, Stouvenakers N, Louvet M, Goffinet G, Thome JP. USE OF CULTURED HEPATOCYTES AS AN ALTERNATIVE METHOD TO STUDY THE EFFECTS OF PCBS ON LIVING ORGANISMS. Toxicology In Vitro 1993;7(4):433-437.

In order to study the mechanism of action of polychlorinated biphenyls (PCBs) several parameters have been monitored in cultured foetal rat and quail hepatocytes. At low concentrations, the PCB mixture tested (Aroclor 1254) did not affect the biological and morphological parameters studied. Above 170 mug/ml, Aroclor induced cytotoxic effects and morphological damage, similar to those that have been observed in vivo, in both animal species (i.e. modification of the endoplasmic reticulum structure, appearance of cytoplasmic vacuoles, alteration of the mitochondrial cisternae). Concentrations as low as 10 muM (3 ppm)

induced cytochrome P450IA1- dependent activities (ethoxyresorufin-O-deethylase, ethoxycoumarin-O-deethylase)in rat hepatocytes. In quail hepatocytes, a very significant induction was observed at concentrations as low as 1 muM. This induction was dependent on both dose and duration of exposure. Testosterone metabolism was not affected by the PCB treatment. These in vitro models are helpful alternatives to in vivo systems for the study of the mechanism of action of PCBs on hepatocytes.

CYTOTOXICITY

28

Lemieux P, Michaud M, Page M. A NEW FORMAZAN AMPLIFIED CLONOGENIC ASSAY FOR CYTOTOXICITY TESTING. Biotechnol Tech 1993;7(8):597-602.

Recently, a tetrazolium salt known as MTT was developed to assess mammalian cell proliferation in vitro. Once reduced by active mitochondrial dehydrogenases it produces insol. formazan crystals. These are usually dissolved with DMSO to give a colorimetric test. The authors took advantage of the insol. formazan crystals prodn. to amplify small colonies which are scored by means of a Biotran III automated colony counter. Throughout this study the authors tested whether or not this method could shorten the tech. time applied to score colonies which have grown either in a T-flask or in soft-agar. Results presented below show that MTT may be used for colony enhancement in soft-agar assays. This amplification method was found to be reproducible and sensitive and was applied to cytotoxicity testing for adriamycin.

29

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

The in vitro cytotoxicity test for estg. the non-ocular irritation dose of ophthalmic solns. was investigated. In the in vitro test, normal human epidermal keratinocytes (NHEK) in a confluent monolayer were incubated for 48 h in a medium with test compds. The concn. of a test compd. which causes a 50% redn. in NHEK viability was detd. as IC50 by MTT colorimetric assay. For comparison, the in vivo rabbit ocular irritation tests were carried out by the std. Draize method. The max. concn., which did not show any ocular

irritation, was detd. as DS0. The results showed the correlation coeff. between the IC50 values and the DS0 values for 19 test compds. to be 0.82. However, the correlation coeffs. for 10 compds., which have IC50 values of less than 300mug/mL, and for 7 alcs. were 0.99. The IC50-DS0 correlation curves obtained could be utilized as the crit. concns. for ocular irritation. These results suggest that the authors' in vitro/in vivo test can est. non-ocular irritation dose of the ophthalmic prepns. in advance of the in vivo tests.

30
Smith LM. EVALUATION OF AN IN VITRO CYTOTOXICITY ASSAY FOR SPECIFIC GROUPS OF CHEMICALS. Diss Abstr Int B 1993, 53(9), 4619; 1991,279 pp.

No abstract.

31
Saito H, Koyasu J, Shigeoka T. CYTOTOXICITY OF ANILINES AND ALDEHYDES TO GOLDFISH GFS CELLS AND RELATIONSHIPS WITH 1-OCTANOL/WATER PARTITION COEFFICIENTS. Chemosphere 1993;27(8):1553-60.

The cytotoxicities of 13 anilines and 10 aldehydes to goldfish scale GFS cells were detd. with the neutral red assay. The sequence of cytotoxicity was based on the concn. of chems. that reduced uptake of neutral red by 50 % (NR50 values). Among a series of aniline derivs., the parent aniline mol. was the least cytotoxic, with potency increasing with the progressive incorporation of chlorine atoms or with the alkyl chain length into the arom. ring structure. Among a series of aldehyde derivs., methanal (formaldehyde) and propanal, which have less than two carbon atoms of the alkyl chains, were more cytotoxic than predicted from the alkyl chain length. The in vitro cytotoxicity of these chems. except for methanal was found to be significantly correlated to their in vivo acute toxicity to guppies. Moreover, NR50 values were significantly correlated with 1-octanol/water partition coeffs. (Pow) excluding the lower aliph. aldehydes.

32
Guenther K, Scharf HP, Puhl W. IN-VITRO TOXICITY TESTING OF CERAMICS AND BONE TRANSPLANTS IN A FIBROBLAST CULTURE MODEL. Biomed Tech 1993;

38(10):249-54.

An established animal fibroblast culture technique used to test chem. reagents for acute unspecified cytotoxicity was modified to develop a reliable and in vitro reproducible model for study of tissue reactions after implantation of biomaterials. Gamma-Irradiated synthetic implants and bob=vine hydroxylapatite did not show any substantial cytotoxicity. In contrast, ethylene oxide-sterilized implant materials showed toxicity.

33
Wataha J, Hanks C, Craig R. THE EFFECT OF CELL MONOLAYER DENSITY ON THE CYTOTOXICITY OF METAL IONS WHICH ARE RELEASED FROM DENTAL ALLOYS.
Dent Mater 1993;9(3):172-6.

The effect of cell d. (no. of cells per unit area of a monolayer culture) on the in vitro cytotoxicity of metal ions which are known to be released from dental materials was investigated. The effects of cell d. (1) may explain previous discrepancies in in vitro tests, (2) may be important in wound healing where cell d. changes over time, and (3) may help clarify the mechanisms of cytotoxicity of metal ions. Balb/c 3T3 fibroblasts were plated at cell densities ranging from 10,000-80,000 cells/cm2 and were exposed to 8 concns. of 10 different metal ions. After 24 h, the succinic dehydrogenase activity and DNA synthesis were measured to quantify the cytotoxic effect. Higher cell densities markedly reduced the sensitivity of these fibroblasts to all metal ions except Al+3 and Zn+2, but the magnitude of the redn. was metal dependent. In addn., the DNA synthesis was inhibited more than the succinic dehydrogenase activity for all metal ions except Zn+2. The unique effect of cell d. on each metal ion supported the hypothesis that the effect was not simply caused by a diln. of the no. of metal ions per cell. Given these results, the effect of cell d. should be carefully selected in in vitro cytotoxicity tests.

34
Ciapetti G, Stea S, Cenni E, Sudanese A, Marraro D, Toni A, Pizzoferrato A. TOXICITY OF CYANOACRYLATES IN VITRO USING EXTRACT DILUTION ASSAY ON CELL CULTURES.
Biomaterials 1994;15(2):92-6.

Comparative cytotoxicity testing of four cyanoacrylate

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

35
Katsnelson B, Privalova L, Sharapova N, Kislitsina N.
A CONTRIBUTION TO THE ESTABLISHMENT OF A BATTERY OF SHORT-TERM TESTS FOR ESTIMATING COMPARATIVE CYTOTOXICITY. Curr Toxicol 1993;1(1):63-9.

This paper considers the main advantages of using ests. of the cytotoxicity of low-sol. particles for phagocytic cells (macrophages in the first place) for predicting their effect on the organism, and for quickly solving some problems of toxicol. hygienic regulation. The need to improve the reliability of these ests. by means of a battery of tests is emphasized. Preference is given to in vitro tests based on the observation of an effect that depends on damage to the cell only or on both damage to the cell and, with the same sign, secondary activation by macrophage breakdown products of the cells in the culture that have not yet been damaged. If comparative ests. of cytotoxicity obtained for a group of substances by different tests from a battery do not agree, the decision should be made, taking into account in vivo cytotoxicity ests. obtained by a cytol. characteristic of a broncho-alveolar lavage performed 24 h after intratracheal administration of the same particles in small doses, special importance being attached to an increase in the lavage. The main methodol. requirements for cytotoxicity tests are considered.

36
Wakuri S, Izumi J, Sasaki K, Tanaka N, Ono H.
CYTOTOXICITY STUDY OF 32 MEIC CHEMICALS BY COLONY

FORMATION AND ATP ASSAYS. Toxicol in Vitro 1993;7(4):517-21.

The cytotoxicity of the first 32 of the 50 chems. listed in the Multicenter Evaluation of In vitro Cytotoxicity (MEIC) program was evaluated by colony formation (BALB 3T3 cells) and ATP assays (HL-60 cells and mouse erythrocytes). Significant correlations (r = 0.90-0.95) were obtained with ID50 values (50% inhibition dose in comparison with the control) of the 23-30 chems. from which such values could be obtained, in erythrocytes vs. HL-60 cells, BALB 3T3 vs. HL-60 cells and BALB 3T3 cells vs. erythrocytes. When ID50 values from colony formation and ATP assays of 9 or 10 chems. were compared with human acute oral LD, human acute lethal blood concn. and mouse oral LD50, close

correlations (r = 0.80-0.97) were seen between data from in vitro and in vivo tests. These results suggest that colony formation and ATP assays are useful for screening chems.

37
Page B, Page M, Noel C. A NEW FLUOROMETRIC ASSAY FOR CYTOTOXICITY MEASUREMENTS IN VITRO. Int J Oncol 1993;3(3):473-6.

Cytotoxicity testing of anticancer drugs requires techniques which are sensitive, reproducible and applicable to large scale testing using automated instruments. These assays are presently performed with end point staining of cell proteins with dyes, viability stains or energy dependent of substrates such as MTT or XTT. Although reliable, these assays are not sensitive enough, too expensive for large scale screening or they use reagents that may be harmful for personnel or equipment. The authors describe, the use of Alamar Blue, a new non fluorescent substrate, which after redn. in living cells, yields a very strong fluorescent product. Using the automated fluorescence plate reader Cytofluor, the authors have evaluated the various parameters such as substrate concn., time and vol. of incubation with respect to linearity and lower limit of detection. The authors found that for a two-h assay, this new non toxic substrate could detect as low as 200 cells per well with a useful measurement range up to 20,000 cells per well. The fluorescent assay is more than ten times as sensitive as the colorimetric assay. When the cytotoxicity of daunorubicin was measured with this assay and compared to the XTT

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

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

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

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

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

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

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

and type of tests that have to be included in a battery

which encompasses a broad spectrum of toxicity levels. Consequently, this study supports the use of a selected battery of tests to evaluate ecotoxicity and suggests its possible importance for screening of biol.-active compds. from natural sources.

41
Bernhardt G, Reile H, Birnboeck H, Spruss T, Schoenenberger H. STANDARDIZED KINETIC MICROASSAY TO QUANTIFY DIFFERENTIAL CHEMOSENSITIVITY ON THE BASIS OF PROLIFERATIVE ACTIVITY. J Cancer Res Clin Oncol 1992;118(1):35-43.

Conventionally in vitro cytotoxicity assays are performed as single-end-point detns. To compensate for the diversity of growth rates among different cell lines in this report the authors describe a computerized kinetic chemosensitivity assay based on quantification of biomass by staining cells with crystal violet. As a prerequisite four human breast cancer cell lines (MDA-MB-231, MCF-7, T-47-D and ZR-75-1) were characterized with regard to estrogen and progesterone receptor content, modal chromosome no. and proliferation kinetics depending on the no. of passages in culture. With prolonged time in culture for ZR-75-1 exposed to various concns. of cisplatinum a dose-related increase in drug effect was obsd. Owing to a correction of the T/C values for the initial cell mass (at the time when drug is added) a sharp distinction between cytostatic and cytocidal drug effects becomes obvious in plots of cor. T/C values vs. time of incubation. The influence of the untreated control on the cor. T/C values and possible time courses of theor. inhibition profiles (reflecting cytostatic, transient cytotoxic or cytocidal drug effects as well as development of resistance) and their relationship to the corresponding growth curves of drug-treated cells are discussed. Chemosensitivity assays with diethylstilbestrol dipropionate, tamoxifen, melphalan, cisplatinum, vinblastine, Adriamycin and 5-fluorouracil prove the theor. considerations to be true for MDA-MB-231, MCF-7, T-47-D and ZR-75-1 human breast cancer cell lines in practice.

42
Northup SJ. CYTOTOXICITY, MUTAGENICITY, AND IMMUNOTOXICITY. Cardiovasc Pathol 1993;

2(3):129S-136S. (45 REFS)

Exceptional and ordinary results in the published literature are reviewed with special emphasis on blood-material interactions. This chapter emphasizes interactions in the blood path, although it is recognized that any device that has contact with internal organs or tissues has contact either directly or indirectly with the blood path. Advances have been made in the evaluation of devices and materials for cytotoxicity and genotoxicity. The principal methods for cytotoxicity, i.e., direct contact, ext. diln., and agar diffusion assays, have been employed extensively throughout industry, government, and academia. They have been developed into std. methods in developed countries and by international organizations. Future directions will entail refinement of these assays, newer automated methods, and improvements in the understanding and hazard assessment of target cell toxicity. Genotoxicity tests, including mutagenicity and clastogenicity, have had widespread applications in testing pure chems. Although the methods have been developed into international stds., there are many deviations and uncertainties in the applications of these stds. and the interpretation of data. In particular, there is considerable confusion about the use of these methods for complex mixts. including the extractables from medical devices. There is no convincing evidence that long-term human clin. use of bioprosthetic or prosthetic devices has resulted in irreversible genotoxic effects. In vitro immunotoxicity testing has been primarily limited to phagocytosis and complement testing. Assays for hypersensitivity and cell killing have been developed but not exploited.

43
Tempel KH, Ignatius A. TOXICOLOGICAL STUDIES WITH PRIMARY CULTURES OF CHICK EMBRYO CELLS: DNA FRAGMENTATION UNDER THE INFLUENCE OF DNASE I-INHIBITORS. Arch Toxicol 1993; 67(5):318-24.

Chicken embryo brain and liver cells in vitro exhibited spontaneous DNA fragmentation as determined by viscometry of alk. cell lysates. Ca2+ and Mg2+ enhanced, while Zn2+, the Ca2+ chelator ethyleneglycolbis (beta-aminoethyl-ether)-N,N,N'-tetraacetic acid (EGTA), spermine and - to a lesser extent - spermidine and Hoechst 33,258 inhibited spontaneous DNA fragmentation. Under the same conditions chromatin condensation, as assessed by

nucleoid sedimentation, increased. Exposure of chicken embryo cells to various genotoxic agents, i.e. doxorubicin, bleomycin, Me methanesulfonate, thiyl radicals, H2O2, UV light, and x-rays, increased DNA fragmentation in a dose dependent manner. Zn2+ or EGTA diminished DNA fragmentation in cells exposed to bleomycin, thiyl radicals, H2O2 and UV light. An apparent sensitization to x-irradn. has been observed in Zn2+ or EGTA-pretreated cells. It is suggested by the present investigations that, with agent specific peculiarities, apoptotic phenomena are implicated when nucleotoxicity is assessed in chicken embryo cells by physico-chem. short-term tests in vitro.

44
Babich H, Borenfreund E. APPLICATIONS OF THE NEUTRAL RED CYTOTOXICITY ASSAY TO RISK ASSESSMENT OF AQUATIC CONTAMINANTS: AN OVERVIEW. ASTM Spec Tech Publ 1993; VOL STP 1179, ISS Environmental Toxicology and Risk Assessment, 1993,215-29.

The neutral red (NR) in vitro cell viability assay is a cytotoxicity test, initially developed for use with mammalian cells to evaluate the acute toxicities of chems. It has been adapted for aquatic ecotoxicity tests by the use of cultured fish cells as the bioindicator system. This assay is based on the binding of neutral red, a weakly cationic, supravital dye, to the lysosomal matrix of viable cells after their incubation with toxic agents. Spectrophotometric quantitation of the extd. dye at 540 nm with a scanning microtiter well reader was shown to be linear with the no. of surviving, undamaged, viable cells. This assay with fish cells as the targets has been applied to five areas of ecotoxicity testing and risk assessment: (1) the ranking of the test agents according to their potencies; (2) the study of metab.-mediated cytotoxicity; (3) the analysis of structure-activity relationships for series of related chems.; (4) the detn. of chemical toxicity as a function of temp.; and (5) the evaluation of chem. interactions as they relate to toxicity.

45
Horwitz JP, Massova I, Wiese TE, Wozniak AJ, Corbett TH, Sebolt-Leopold JS, Capps DB, Leopold WR. COMPARATIVE MOLECULAR FIELD ANALYSIS OF IN VITRO GROWTH INHIBITION OF L1210 AND HCT-8 CELLS BY SOME PYRAZOLOACRIDINES. J Med Chem 1993; 369(230):3511-16.

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

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

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

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

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

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

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

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

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

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

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

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

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

50
Carfagna MA, Held SD, Kedderis GL. FURAN-INDUCED CYTOLETHALITY IN ISOLATED RAT HEPATOCYTES: CORRESPONDENCE WITH IN VIVO DOSIMETRY. Toxicol Appl Pharm 1993;123(2):265-273.

Furan, a rodent hepatotoxicant and hepatocarcinogen, produced incubation time- and concentration-dependent decreases in the glutathione (GSH) content and viability of freshly isolated F-344 rat hepatocytes in vitro. Since furan itself did not significantly react with GSH, these data indicate the formation of a reactive metabolite of furan in hepatocyte suspensions. Treatment of the hepatocyte suspensions with the cytochrome P450 inhibitor 1-phenylimidazole delayed GSH depletion but did not alter furan-induced (4 to 12 mm) cytolethality. The furan concentrations required to produce measurable hepatocyte cytolethality in vitro within 6 hr (4 to 12 mm) were several orders of magnitude greater than the predicted maximal liver concentrations of furan in vivo following hepatotoxic doses. In order to study the mechanisms involved in the cytolethality of furan toward hepatocytes in vitro at concentrations relevant to hepatotoxicity in vivo, a hepatocyte suspension/culture system was developed that utilized furan concentrations and incubation times similar to hepatic dosimetry in vivo. Freshly isolated rat hepatocytes in suspension (in Williams' Medium E) were incubated with furan (2 to 100 muM) for 1-4 hr and placed in culture, and viability was determined after 24 hr by lactate dehydrogenase release. Furan produced cytolethality (5 to 70%) and modest GSH depletion in an incubation time- and concentration-dependent manner. Both GSH depletion and cytolethality induced by furan were prevented by 1-phenylimidazole and enhanced by acetone pretreatment of the rats. These data show that oxidation of furan by cytochrome P450 is required for GSH depletion and cytolethality, indicating that a reactive metabolite is involved in cell death. The results of this study underscore the importance of using in vivo toxicant concentrations and exposure times for in vitro mechanistic studies of chemically induced cytolethality.

51
Bjerredgaard HF. ELECTROPHYSIOLOGICAL MEASUREMENTS OF A TOAD RENAL EPITHELIAL CELL LINE (A6) AS AN ASSAY TO EVALUATE CELLULAR TOXICITY IN VITRO. Toxic In Vitro

1993;7(4):411-415.

An established epithelial cell line (A6) from toad kidney was used to study in vitro cytotoxicity. When grown on permeable support, A6 cells form a monolayer epithelium with a high electrical resistance and a transepithelial potential. These two easily measured electrophysiological endpoints showed a dose-related decrease after exposure of the cells for 24 hr to 21 selected chemicals. It was demonstrated that both transepithelial potential and transepithelial resistance correlated well with acute cytotoxicity data obtained using human lymphocytes and with calculated human lethal dose values. The polarity of the epithelial cells was demonstrated by specific chemicals that targeted the basolateral membrane. The results show that electrophysiological measurements of A6 epithelia could be used as a general cell model to study cytotoxicity and as a specific model to evaluate toxic affects on tight epithelia.

52
Van de Water C, Van Dura EA, Van der Stap J G MM, Brands R, Boersma W JA. RAPID IN VITRO MICRO-CYTOTOXICITY TESTS FOR THE DETECTION AND QUANTITATION OF NEUTRALIZING ANTIBODIES TO BOTH VIRUSES AND TOXINS. J Immun Meth 1993;166(2):157-164.

A generally applicable method was developed for the rapid and quantitative detection of both toxin and virus neutralizing antibodies. The method was optimized for three different biological agents, i.e., Shigella toxin, influenza viruses (A/Beying, A/Taiwan and B/Yamagata) and Chikungunya virus. The in vitro micro-cytotoxicity tests developed for the detection and quantitation of neutralizing antibodies are based on the inhibition of the virus- or toxin-induced cytotoxic effect by antibodies. As a result of the cytotoxicity, infected cells are no longer attached to the solid phase and can be easily removed. Thereafter, the proteins of the remaining living cells are stained. After removing the excess dye, the remaining dye is dissolved and the absorbance values are measured. The neutralization titers are determined from the absorbance values. Since the tests are performed in wells of microtiter plates, the in vitro micro-cytotoxicity tests are less laborious and consume less reagent in comparison with classical neutralization tests.

53
Weiss MT, Sawyer TW. CYTOTOXICITY OF THE MEIC TEST CHEMICALS IN PRIMARY NEURONE CULTURES. Toxicology In Vitro 1993;7(5):653-667.

50 Multicentre Evaluation of In Vitro Cytotoxicity (MEIC) test chemicals were assayed for cytotoxicity in primary cultures of chick embryo forebrain neurones using the MTT and neutral red assays. The neutral red assay was consistently more sensitive to chemical toxicity; however, both assays were equally predictive when compared with in vivo toxicity data obtained from the Registry of Toxic Effects of Chemical Substances. High correlations were obtained when comparing the in vitro data with ip 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 chemicals, the cytotoxicity of some compounds was dependent on the degree of differentiation of the neurone cultures, suggesting that this culture system may not only be sensitive to the basal cytotoxicity of chemicals, but also to toxic effects specific to the specialized differentiated functions of the central nervous system.

54
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):1511-1518.

Cytotoxicity testing of non-hydrosoluble chemicals offers a major problem because cells are always cultured in aqueous 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 containing the test chemical, solubilized in paraffin. After 24 h the total protein content was measured. The relative toxicity is expressed by the EC50. This is the concentration of test chemical 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 quantitative cytotoxicity data of lipophilic chemicals towards cultured fish cells.

55
Calleja MC, Persoone G, Geladi P. COMPARATIVE ACUTE TOXICITY OF THE FIRST 50 MULTICENTRE EVALUATION OF IN VITRO CYTOTOXICITY CHEMICALS TO AQUATIC NON-VERTEBRATES. Archives of Environmental Contamination and Toxicology1994;26(1):69-78.

The acute toxicity data of the first 50 chemicals of the Multicentre Evaluation of In Vitro Cytotoxicity (MEIC) programme is compared for three "cyst-based toxicity tests" (Artoxkit M with Artemia salina, Streptoxkit F with Streptocephalus proboscideus, and Rotoxkit F with Brachionus calyciflorus), and two other tests (the Daphnia magna and the Photobacterium phosphoreum Microtox tests) commonly used in ecotoxicology. The difference in sensitivity for the 50 chemicals was as high as 19 orders of magnitude (on a molecular weight basis) between the most and least sensitive species. Generally, a similar toxicity ranking of the 5 test species was found for most of the chemicals and the interspecies correlations were high. Results from Principal Component Analysis (PCA) and cluster analysis indicated that the groupings are not related to a clear and defined chemical structure. However, the loading plot of the first two principal components may aid in selecting the minimum number and type of tests that have to be included in a battery which encompasses a broad spectrum of toxicity levels. Consequently, this study supports the use of a selected battery of tests to evaluate ecotoxicity and suggests its possible importance for screening of biologically-active compounds from natural sources.

56
Hurbankova M, Tilkes F. USE OF THE TEST FOR ESTIMATION OF LDH ACTIVITY AS ONE PARAMETER FOR DEMONSTRATING CYTOTOXICITY OF FIBROUS DUST. Prac Lek 1993; 45(4):149-153.

The unfavorable effect or asbestos fibres on the

organism followed to the use of substitute materials supposedly eliciting lower biological response. The effects of fibrous dust in the in vitro or in vivo conditions have usually been evaluated in laboratory animals as test systems. In vitro tests have been given preference over the last years due to the use of considerably lower quantities of animals. Alveolar macrophages have been given priority in cell cultures for demonstrating cytotoxicity of fibrous and non-fibrous dusts in view of the fact that these cells are the first to come into contact with the inhaled noxa. The authors used the in vitro determination or lactate dehydrogenase (LDH) activity or guinea pig alveolar macrophages after 20-hour exposure to fibrous dusts (crocidolite, wollastonite from China, wollastonite from the USA, Supelco, Dolanit) at doses of 100, 200 and 300 muglveolar macrophages. The results were compared with unexposed control and with non-fibrous dusts (corundum - relatively inert, and DQ12 - a highly toxic dust). The decreasing order of toxicity was displayed by DQ12, asbestos-crocidolite, wollastonite - China, wollastonite - USA, corundum, Dolanit and Supelco. LDH values followed a statistically significant concentration gradient in all types of the examined samples with the exception of Supeko and Dolanit, where the effect were not related to the dose of fibres.

57
Romert L, Jansson T, Jenssen D. THE CYTOTOXICITY OF 50 CHEMICALS FROM THE MEIC STUDY DETERMINED BY GROWTH

INHIBITION OF ASCITES SARCOMA BP8 CELLS: A COMPARISON WITH ACUTE TOXICITY DATA IN MAN AND RODENTS. Toxicol Lett 1994;71(1):39-46.

In this study, 50 chemicals selected on the basis of existence of particularly reliable human toxicity data were screened in a cytotoxicity test involving inhibition of the growth of Ascites Sarcoma BP8 cells. These test results are part of an international validation program, the Multicenter Evaluation of In Vitro Cytotoxicity (MEIC), the aim of which is to recommend batteries of in vitro tests to be used for prediction of human toxicity. The cytotoxicities (expressed as the concentrations causing 50% inhibition of cell growth) were compared to acute toxicity data in humans (LDL0) and rodents (LD50), showing the best correlation to rodent data. The results are discussed in relationship to what is usually referred to as basal

cytotoxic mechanisms as a cause of in vivo toxicity. It could be concluded that the predicted results on the basis of mechanistic reasoning were not always obtained.

58
Wilheilm KP, Samblebe M, Siegers CP. QUANTITATIVE IN VITRO ASSESSMENT OF N-ALKYL SULPHATE-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 number of sodium salts of N-alkyl sulphates 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 morphology criteria 24 h after dosing. A linear proportionality between keratinocyte number 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. Concentrations resulting in 50% inhibition of NR uptake (IC-50) ranged from 0.15 mmol (sodium lauryl sulphate, C12) to 1.23 mmol (sodium octyl sulphate, 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 compounds (300 microliters of 20 mmol aqueous solution) 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 colour reflectance measurements). For the test substances, however, the sensitivity of the in vitro system was between 10 and 100 times higher than that observed in human skin in vivo.

59
Yang W, Acosta D. CYTOTOXICITY POTENTIAL OF SURFACTANT MIXTURES EVALUATED BY PRIMARY CULTURES OF RABBIT CORNEAL EPITHELIAL CELLS. Toxicol Lett 1994;70(3):309-18.

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

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

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

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

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

2-hydroxy-3-alkyl-1,4-NQs, as well as some amino derivatives, were evaluated with the neutral red cytotoxicity assay, using BALB/c mouse 3T3 fibroblasts. As compared to the unsubstituted 1,4-NQ: (i) Substitution at the 2 position reduced toxicity, with the extent of reduction following the sequence, hydroxyl alkyl group. (ii) Substitution with C2-C5 alkyl groups at position 3 enhanced toxicity. As noted with the n-alkyl-1,4-NQs, increasing the chain length of the 2-hydroxy-3- alkyl-1,4-NQs did not appreciably change potency of the test agent. (iii) Substitution with amino groups at position 3 had little effect on cytotoxicity. Some differences in cytotoxicity of specific test agent were noted between the 3T3 fibroblasts and isolated rat hepatocytes, as reported in the literature.

62
Zahn T, Hauck C, Braunbeck T. CYTOLOGICAL ALTERATIONS IN FISH FIBROCYTIC R1 CELLS AS AN ALTERNATIVE TEST SYSTEM FOR THE DETECTION OF SUBLETHAL EFFECTS OF ENVIRONMENTAL POLLUTANTS A CASE-STUDY WITH 4 CHLOROANILINE. Braunbeck, T., W. Hanke and H. Segner (ED.). Fish: Ecotoxicology and Ecophysiology; International Symposium, Heidelberg, Germany, September 25-27, 1991. XVII+418P. VCH Vellagsgesellschaft MBH: Weinheim, Germany; VCH Publishers, Inc.: New York, New York, USA. ISBN 3-527-30010-4; ISBN 1-56081-704-6. 1993; 0(0):103-126.

No abstract.

63
Lenz D, Segner H, Hanke W. COMPARISON OF DIFFERENT ENDPOINT METHODS FOR ACUTE CYTOTOXICITY TESTS WITH THE R1-CELL LINE. Braunbeck, T., W. Hanke AND H. Segner (ED.). Fish: Ecotoxicology and Ecophysiology; International Symposium, Heidelberg, Germany, September 25-27, 1991. XVII+418P. VCH Verlagsgesellschaft MBH: Weinheim, Germany; VCH Publishers, Inc.: New York, New York, USA. ISBN 3-527-30010-4; ISBN 1-56081-704-6. 1993;0(0):93-102.

No abstract.

64
Rusche B, Kohlpoth M. THE R1-CYTOTOXICITY TEST AS A REPLACEMENT FOR THE FISH TEST STIPULATED IN THE GERMAN

WASTE WATER ACT. Braunbeck, T., W. Hanke AND H. Segner (ED.). Fish: Ecotoxicology and Ecophysiology; International Symposium, Heidelberg, Germany, September 25-27, 1991. XVII+418P. VCH Verlagsgesellschaft MBH: Weinheim, Germany; VCH Publishers, Inc.: New York, New York, USA. ISBN 3-527-30010-4; ISBN 1-56081-704-6. 1993;0(0):81-92.

No abstract.

65
Fortunati E, Debetto P, Borella S, Bianchi V.
INHIBITION OF CELL GROWTH AND ALTERATION OF CYTOSOLIC CALCIUM LEVELS IN THE CYTOTOXICITY EVALUATION OF NINE MEIC CHEMICALS. Toxicology in Vitro 1993;7(4):511-516.

Inhibition of cell growth was compared in V79 and HeLa cell cultures treated for 60 hr with nine of the first 10 MEIC chemicals; FeSO4 could not be tested because it produced artefacts. Whereas the IC50 of digoxin was at least three orders of magnitude lower in HeLa cells, all the other chemicals were almost equally toxic in the two cell lines. The IC50 values showed good correlation with the in vivo human toxic concentrations, but the correlation was better with HeLa cells, which allowed the species-related sensitivity to digoxin to be detected. The effects of acute exposures to the same compounds on the cytosolic free Ca2+ of PC12 cells, a neurosecretory cell line derived from a rat phaeochromocytoma, were measured fluorometrically by the fura-2 method. Amitriptyline, methanol, ethanol and isopropanol increased resting (Ca2+)i, both in the presence of extracellular Ca2+ and, to a lesser extent, in Ca2+-free medium. Diazepam, digoxin and ethylene glycol were effective exclusively in the former condition. The changes of resting (Ca2+)i appear to be sensitive indicators of early cytotoxicity induced by different toxic chemicals.

DENTAL TOXICITY
66
Watts A, Paterson RC. DEVELOPMENT AND HARMONISATION OF THE NORMS AND PARAMETERS IN BIOLOGICAL TESTING PROTOCOLS DESIGNED FOR ROOT CANAL SEALING MATERIALS WITHIN THE EUROPEAN COMMUNITY. Int Dent J 1993; 43(6):599-605.

The EC has mounted the BIOMED 1 as a specific programme to facilitate cooperative research programmes in

identified areas of interest throughout Europe. They have recently announced support under this programme for a joint initiative designed to evaluate in vitro and in vivo methods of biological testing of root canal sealing materials. This proposal was prepared by a group of research workers in the field. It provides a summary of the current suggested protocols for the biological testing of dental materials and then provides a detailed protocol of the studies to be carried out in the joint programme which commenced in January 1993. The authors are publishing the proposal to invite comments from colleagues and interested parties.

DERMAL TOXICITY

67
Moody RP. IN VITRO DERMAL ABSORPTION OF PESTICIDES: A CROSS-SPECIES COMPARISON INCLUDING TEST SKIN. J Toxicol, Cutaneous Ocul Toxicol 1993;12(2):197-202.

The present study reports the use of the authors' automated in vitro dermal absorption (AIDA) procedure developed inhouse as a potential alternative to in vivo testing. Finite-dose AIDA studies were conducted with the pesticides DEET, 2,4-D, Diazinon, and DDT, these compds. being chosen for their wide range of lipophilicities. Absorption (percentage recovery in receiver soln.) in the human-derived tissue-cultured skin, Test skin, was similar to that in pig skin for three of the four test compds. Test skin was 2.5 times more permeable than pig skin to 2,4-D. Continuous-dose AIDA studies conducted with the swimming pool stabilizer, cyanuric acid (CYA), demonstrated minimal CYA absorption through rat, hairless guinea pig, human, and Test skin. Total cumulative absorption of CYA by 24 h in Test skin and human skin was 0.02 mug CYA/cm2 in both cases.

68
Ruland A, Kreuter J, Rytting JH. TRANSDERMAL DELIVERY OF THE TETRAPEPTIDE HISETAL (MELANOTROPIN (6-9)): II. EFFECT OF VARIOUS PENETRATION ENHANCERS. IN VITRO STUDY ACROSS HUMAN SKIN. Int J Pharm 1994; 103(1):77-80.

The percutaneous absorption of the tetrapeptide hisetal as well as the effect of various penetration enhancers

on the permeation of hisetal across human skin was evaluated by in vitro methods in Franz cells. The

passive permeability coeff. for hisetal was found to be 0.93 .times. 10-5 cm h-1. In comparison to the permeation across hairless mouse skin (findings of part 1) the permeability coeff. was decreased by a factor of 6. Enhancer treatment led to an increase in permeability by a factor of maximally 6 (OA). The relatively new permeation enhancers DDAA and DAIPD were found to increase the permeation of hisetal to similar extents as Azone. In order to show that the decreased enhancer effects were not due to the exptl. design, a second set of investigations was carried out. Whereas drug and enhancer were applied simultaneously during the first set, in the second set of investigations the human skin was pretreated with neat enhancer for 3 h. The results from this second set did not differ significantly from those of the first set. Consequently, these results combined with the findings of part I (hairless mouse skin penetration) clearly demonstrated that hairless mouse skin is influenced by enhancer treatment in an exaggerated manner.

69
Pearse AD, Edwards C. HUMAN STRATUM CORNEUM AS A SUBSTRATE FOR IN VITRO SUNSCREEN TESTING. Int J Cosmet Sci 1993; 15(6):234-44.

In this study, the authors described the construction of a dedicated, inexpensive and portable instrument designed to evaluate sunscreens throughout the UVB and UVA range (290-400 nm). Both Transpore and an alternative substrate of readily obtainable human stratum corneum have been used as substrates on to which to spread the test products. The transmission of UVR through the substrate of stratum corneum was greater than through Transpore tape. Both substrates demonstrated a good correlation with in vivo or expected sun protection factor (SPF) results. However, in 10 out of the 11 sunscreens studied, the comparison of the 2 substrates demonstrated that the predicted SPF using Transpore tape was consistently higher than that using human stratum corneum. One sunscreen tested was alc. based and as such was not suitable to test on Transpore tape. Predictions of SPF from products with SPF values less than 20 were not significantly different between substrates. However, product SPF values of >20 demonstrated that Transpore tape overestd. the 'true' SPF. It is postulated that use of human stratum corneum in in vitro SPF testing systems more closely resembles that of human skin in vivo than does Transpore tape with regard to spreading and

absorption of the potential sunscreen product.

70
Rahman MS. IN VITRO METHOD DEVELOPMENT AND INVESTIGATION OF THE PERMEATION OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN THROUGH HAIRLESS MOUSE AND HUMAN SKINS. Diss Abstr Int B 1992; 53(2):794.

No abstract.

71
European Centre, for Ecotoxicology, and Toxicology of Chemicals. PERCUTANEOUS ABSORPTION. Tech. Rep. ECETOC 1993; 20:80 pp.

A review with several refs. on factors influencing percutaneous absorption, methods for measuring percutaneous absorption, presentation of results, relevance to man of animal in vivo or animal and human in vitro data, exposure assessment, hazard and risk assessment of chem. contaminated skin, and recommendations.

72
Whittle E, Basketter DA. THE IN VITRO SKIN CORROSIVITY TEST. DEVELOPMENT OF METHOD USING HUMAN SKIN. Toxicol in Vitro 1993;7(3):265-8.

To meet the EEC regulations for classification, packaging, labeling and transportation, many new substances have to be tested for their potential cutaneous toxicity. At present, this is assessed in an in vivo rabbit dermal test. The in vitro skin corrosivity test has been developed to identify those substances which would cause a corrosive response in an in vivo test, thereby alleviating the need to assess corrosive substances in an in vivo test. The method, which uses ex vivo rat skin, is based on the observation that corrosive substances cause a significant redn. in the elec. resistance of skin. The present study investigated the possibility of using human skin in the in vitro skin corrosivity test to provide a means of direct assessment of effect in humans. Twelve substances were assessed in this model. The transcutaneous elec. resistance (TER) following treatment with distd. water is essentially the same as that for untreated skin. Of the six prepns. that would be formally classified as corrosive by the EEC, on the

basis of a 4-h rabbit covered patch test, all caused a sharp fall in the TER for each specimen of human skin on which they were tested. On the basis of these data, the TER threshold for detn. of potential corrosivity was judged to be 11.0 kohms/disk of skin. For the five prepns. which would be classified as irritant or would not require a label, the redn. was usually much less marked. Continuing work in this lab. is directed towards an assessment of a much wider range of chems. using the human kinetics in vitro model, ultimately with a view to its use as a method of detecting those substances which may be corrosive to the skin of humans, leading to their classification and appropriate labeling.

73
Hirvonen J, Sutinen R, Paronen P, Urtti A. TRANSDERMAL PENETRATION ENHANCERS IN RABBIT PINNA SKIN: DURATION OF ACTION, SKIN IRRITATION, AND IN VIVO/IN VITRO COMPARISON. Int J Pharm 1993;99(2-3):253-61.

Irritation of the skin by chem. penetration enhancers may limit the use of these compds. in transdermal drug delivery. Biodegradable enhancers like dodecyl N,N-dimethylaminoacetate (DDAA) have been synthesized previously to decrease the duration of action and toxicity of the enhancers. The authors studied the reversibility and extent of penetration enhancement and skin irritation by DDAA, Azone, and n-dodecanol in rabbit pinna skin using timolol and propranolol as penetrants. Also, in vitro and in vivo permeabilities of the drugs with and without enhancers were compared. Drug concns. in diffusion chambers and rabbit plasma were detd. using HPLC and radio receptor assay, resp. Skin irritation was measured with a chromameter, DDAA and Azone caused approx. equal transdermal penetration enhancement of model drugs in vitro but the potency of n-dodecanol was lower. In vivo, Azone was the most irritating enhancer in rabbit pinna skin. Both enhancer effects and skin irritation by DDAA were reversed in 4 days, while the effects of Azone and n-dodecanol lasted longer. Thus, it is possible to affect the duration of skin alteration by enhancer design. Propranolol was more irritating than timolol in rabbit pinna skin in vivo. Percutaneous permeability of propranolol in vivo, calcd. from pharmacokinetic parameters, was considerably greater than its in vitro permeability coeff. In contrast, in vitro and in vivo permeability coeffs. of timolol were comparable. The increased permeation of propranolol in vivo may be due to skin

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

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

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

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

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

.times. 105 per well. These data show that the cell no. of 1 .times. 104 per well in a 96-well flat-bottomed microculture plate and an assay at 4 days are the suitable exptl. conditions for the measurement of DNA, RNA, and protein syntheses.

76
Scott RC, Carmichael NG, Huckle KR, Needham D, Savage T. METHODS FOR MEASURING DERMAL PENETRATION OF PESTICIDES. Food Chem Toxicol 1993; 319(7):523-9. (16 REFS)

The quantitation of percutaneous absorption of pesticides is required as part of the registration, re-registration or hazard assessment process. There is a paucity of regulatory guidelines in this area. This paper presents three protocols that can be used to quantitate percutaneous absorption, primarily as a result of continuous skin exposure over a period equiv. to a working day (8 h). A rat in vivo protocol, an in vitro protocol and a human in vivo protocol are described. None of these protocols is considered to be ideal and/or to represent a preferred method. The final choice of protocol must take into account the toxicity and physicochem. properties of the test molecular as well as cost and resource/tech. ability. Nevertheless, the protocols described allow percutaneous absorption to be quantitated, and it is believed that, if adopted, they will prove useful in the regulatory and research areas for the acquisition of data under std. defined conditions.

77
Ashby J, Hilton J, Dearman RJ, Callander RD, Kimber I. MECHANISTIC RELATIONSHIP AMONG MUTAGENICITY, SKIN SENSITIZATION, AND SKIN CARCINOGENICITY. Environ Health Perspect 1993;101(1):62-67.

The relationship between electrophilicity or mutagenicity and a chemical's skin sensitizing properties and skin carcinogenicity was evaluated. Skin sensitization was assessed by exposing the ears of CBA/Ca-mice to test chemicals on 3 successive days. Five days after the start of exposure, the animals received tritiated thymidine and 5 hours thereafter were examined for lymphocyte proliferation in the pooled auricular lymph nodes of the treated ears. Mutagenicity was evaluated using the Salmonella bioassay. Of the 20 mutagens tested, 14 were positive

in the local lymph node assay, including seven known skin carcinogens. The lack of sensitizing activity in the other mutagens was attributed to extremes of intrinsic reactivity, high water solubility and reduced dermal translocation, and inappropriate dermal metabolism. Two agents, recognized as nonmutagens in the Salmonella assay, were found to be in-vitro clastogens. This indicated that mutagenicity instead of assay activity is the main stimulus for electrophilic sensitization and, consequently, skin carcinogenicity. The authors state that common but individually distinct structure activity relationships determine genotoxicity, and skin sensitization and carcinogenicity.

78
Scott RC, Carmichael NG, Huckle KR, Needham D, Savage T. METHODS FOR MEASURING DERMAL PENETRATION OF PESTICIDES. Food Chem Toxicol 1993;31(7):523-529.

Recommended protocols for measuring dermal penetration of pesticides were reviewed. The procedures which were intended to determine the amount of percutaneous absorption expected to occur over a working day's exposure consisted of applying the test pesticide to the shaved backs of rats, to human or animal skin specimens mounted on diffusion cells, and applying the test chemical to the skin of human volunteers. Detailed descriptions of the procedures were provided. The advantages and disadvantages of each protocol were considered. The in-vivo rat protocol is the classical technique for investigating percutaneous absorption. Rats are readily available, convenient to handle, and are the species most widely used for toxicology and metabolism studies. The disadvantage is that rat skin does not precisely mimic the permeability of human skin. The use of rat data can result in a significant overestimation of probable human skin absorption. In-vitro diffusion cell techniques offer the advantage of using human skin. If animal skin is used, the methodology results in fewer animals being expended that in in-vivo studies. Studies using human volunteers provide the most relevant data for risk assessment. Sophisticated techniques will usually be required because the test agent may undergo extensive metabolism after being absorbed. Studies in human volunteers are limited to nontoxic chemicals since ethical considerations preclude testing toxic pesticides.

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

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

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

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

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

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

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

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

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

No abstract.

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

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

No abstract.

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

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

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

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

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

No abstract.

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

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

absorption, while PA slightly decreased both PO and PNP absorption. This study demonstrated that the absorption data from a single-parent compound or metabolite alone were not adequate to determine the rate of PA absorption from a mixture. Chemical impurities in PA may significantly affect the interpretation of transdermal absorption, metabolism, and risk estimation studies.

87
Bloom E, Sznitowska M, Plannsky JR, Maibach HI. IN VITRO TESTS FOR IRRITANTS: QUALITATIVE DIFFERENCES IN BIOLOGICAL PROPERTIES. In Vitro Toxic 1993; 6(3):171-185.

The irritancy/toxicity of compounds is often evaluated in vitro using simple tests with one-dimensional endpoints such as inhibition to cell growth. The purpose of this study was too examine differences between irritants based on the following qualitative criteria: (1) rate of development of toxic effect on growth (rapid or delayed/progressive) (2) dose response correlation of morphologic and growth changes (3) qualitative classification of morphologic changes and (4) rate of appearance of morphologic effects (rapid or delayed/progressive). Sodium lauryl sulfate (SLS), Triton X-100, phenol, ethylphenyl propiolate (EPP), tetradecanoyl phorbol acetate (PMA) were studied. SLS, Triton X-100, and PMA were rapid onset, <1 hr, in their growth toxicity effects whereas phenol and EPP were progressive over 18 hours in their toxic growth effects. Morphologicaly, SLS, Triton X-100 and phenol showed microscopically visible effects at roughly the same concentrations as required for growth inhibition, where as EPP and PMA did not. SLS and Triton X-100 caused similar but not identical morphologic changes, with prominent cell rounding and cell shrinkage. Phenol, EPP and PMA caused little or no morphological changes around the I50 for growth inhibition. Higher doses caused morphologically distinct changes for each irritant. SLS and Triton X-100 caused morphological changes in less than 1 hour whereas phenol, EPP and PMA caused progressive morphological changes over 18 hours, when added in sufficient concentrations to induce morphological changes.

88
Keeble VB, Correll L, Ehrich M. EVALUATION OF KNIT GLOVE FABRICS AS BARRIERS TO DERMAL ABSORPTION OF

ORGANOPHOSPHORUS INSECTICIDES USING AN IN VITRO TEST SYSTEM. Toxicology 1993;81(3):195-203.

Cotton and synthetic knit glove fabrics in combination with an in vitro skin model were used to examine the capability of fabric to decrease the dermal absorption of the organophosphorus insecticides azinphos-methyl, paraoxon, and malathion. Capability for inhibition of acetylcholinesterase was determined in samples of media taken from under the skin barrier after the skin model, with or without fabric protection, had been exposed to the test compounds for 4 h. Acetylcholinesterase inhibitions caused by the direct addition of organophosphorus insecticide to the media were also included in the comparison. Results indicated that the skin model system alone had some capability to serve as a carrier to the transfer of organophosphates. Fabric covering used on the test model increased the barrier between insecticide application and resultant acetylcholinesterase inhibition. The all-cotton, 7-cut knit was especially effective in preventing the absorption of azinphos-methyl, as this organophosphorus insecticide had no capability to cause acetylcholinesterase inhibition when this fabric was used to protect the skin model. Knit glove materials of 100% cotton were demonstrated to be effective in preventing the absorption of paraoxon and malathion. These studies indicate that an in vitro model system can be used in combination with fabrics to study the relationship between clothing and skin as barriers to the absorption of organophosphorus insecticides.

89
Jacobs RR, Phanprasit W. AN IN VITRO COMPARISON OF THE PERMEATION OF CHEMICALS IN VAPOR AND LIQUID PHASE THROUGH PIG SKIN. Am Ind Hyg Assoc J 1993; 54(10):569-575.

This study used pig skin to compare vapor and liquid permeation of benzene, n-butanol, and toluene in vitro. Vapors of radio-labeled chemicals were generated by passing purified air through two saturators in series containing the labeled chemical. The generated vapor was directed into the donor compartment of a modified liquid permeation cell. For liquid permeation experiments, neat chemicals were dosed directly on the surface of the skin. The variability of the generated concentrations for the vapor phase of each chemical ranged from 3-7%. The mean flux of the liquid chemicals was significantly higher than those of the vapor phase.

There was no significant difference in the flux of the individual chemicals in the liquid phase. In the vapor phase test, the flux of toluene and benzene were not significantly different; however, for n-butanol the flux was significantly lower than that for either benzene or toluene.

90
de Haan P, Heemskerk AE, Gerritsen A, de Boer EM, Sampat S, van der Raaij-Helmer EM, Bruynzeel DP. COMPARISON OF TOXICITY TESTS ON HUMAN SKIN AND EPIDERMOID (A431) CELLS USING FREE FATTY ACIDS AS TEST SUBSTANCES. Clin Exp Dermatol 1993;18(5):428-33.

Several in-vivo methods can be used to determine the ability of chemical compounds to induce skin irritancy. In this study we estimated in vivo the capacity of several free fatty acids to induce skin irritancy and compared the results with those found in in vitro tests. Skin irritancy induced by free fatty acids (chain lengths: C6, C7, C9, C10, C11, C13 and C18) was evaluated in humans by means of laser-Doppler flowmetry (LDF) and visual scoring (VS). Both methods demonstrated that the toxic effect of free fatty acids determined by LDF and VS increased from C6 through C11 and decreased again for C13 and C18. The cytotoxic effect of these free fatty acids on cells was measured in vitro by incubation of human epidermoid cells (A431) with these compounds. It was determined by measuring: (a) the number of dead cells by inclusion of Trypan blue (TB); and (b) the number of living cells by mitochondrial metabolism of 3-[4,5-dimethylthiazole-2-yl]-2,5 diphenyltetrazolium bromide (MTT). The LD-50 concentrations decreased from C6 through C11 in both in-vitro assays. The results of the in-vitro assays for C13 and C18 both demonstrated a discrepancy. The cytotoxic effect of the free fatty acids expressed as LD-50 values, determined after 20 min with the TB assay, was seen at higher concentrations than after incubation for 18 h (MTT assay). From the results it was concluded that C13 in particular affected skin blood flow. We also determined correlation coefficients between the in-vivo and in-vitro methods. When C13 is excluded these coefficients ranged from -0.77 to -0.92.

91
Begg AC, Russell NS, Knaken H, Lebesque JV. LACK OF CORRELATION OF HUMAN FIBROBLAST RADIOSENSITIVITY IN

VITRO WITH EARLY SKIN REACTIONS IN PATIENTS UNDERGOING RADIOTHERAPY. Int J Radiat Biol 1993;64(4):393-405.

Fibroblasts from breast cancer patients were obtained as outgrowths in vitro from punch biopsies and their radiosensitivity tested in early passages. Skin erythema reactions in the same patients were also measured, as degree of redness using reflectance spectrophotometry. Measurements were taken before and during a 4-week radiotherapy treatment with electrons to the thoracic wall. Of 59 biopsies studied, radiosensitivity and erythema were concurrently studied in 32. I 24, evaluable data from both clinic and laboratory were obtained. A population growth assay in 96-well plates, using absorption of sulphur rhodamine B as the stain for cell numbers, showed good agreement with the colony-formation assay. Plating efficiencies and growth rates in the colony assay were higher using human serum in place of foetal calf serum. Cell survival curves with human serum were mostly exponential with little shoulder. The parameters of survival at 2 Gy (SF2) and the dose required to give 10% survival (D10) were used in the correlations with clinical data; these were 0.25 +/- 0.09 and 3.03 +/- 0.50 Gy, respectively. There was a strong correlation between these two survival curve parameters (r = 0.98). Skin redness was found to linearly increase with time during radiotherapy. The slope of the increase differed markedly from patient to patient, with a range of a factor approx. 10. No correlation was found between SF2 and erythema response in the 24 evaluable patients (r = 0.13, p > 0.5). A similar lack of correlation was found using D10 as the radiosensitivity parameter (r = 0.12, p > 0.5). These data indicate that fibroblast radiosensitivity measured in vitro cannot be used to predict erythema reactions to radiotherapy in breast cancer patients.

92
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.

Recent advances in techniques for culture of human skin cells have led to their potential for use as in vitro models for skin irritation testing to augment or replace existing rabbit skin patch tests. Our 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 commercial 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 chemicals with the exception of acids and alkalis, but did not correlate with skin irritation to surfactant-containing product formulations. Cultures of human skin equivalents were evaluated as potential models for measurement of responses to test materials that could not be measured in the keratinocyte/neutral red assay. We 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 experiments 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 preclinical skin irritation assessments, as alternatives to rabbits, for at least certain classes of test substances.

DEVELOPMENTAL TOXICITY

93
Schmid BP, Honegger P, Kucera P. EMBRYONIC AND FETAL DEVELOPMENT: FUNDAMENTAL RESEARCH. Reprod Toxicol 1993;7(Suppl 1):155-164. (35 REFS)

A review which focuses not only on the rat whole embryo culture system but also on modifications that were undertaken for the in vitro chick embryo system and the aggregate cultures of fetal rat brain cells. Today these tests cannot replace the existing in vivo developmental toxicity tests. They can, however, be used to screen chemicals for further development or

further testing. In addition, these in vitro tests provide valuable information on the mechanisms of developmental toxicity and help to understand the relevancy of findings for humans. In vitro systems, combined with selected in vivo testing and pharmacokinetic investigations in animals and humans, can thus provide essential information for human risk assessment.

94
Schmid BP, Honegger P, Kucera P. EMBRYONIC AND FETAL DEVELOPMENT: FUNDAMENTAL RESEARCH. Reprod Toxicol 1993;7(Suppl 1):155-164. (35 REFS)

A review which focuses not only on the rat whole embryo culture system but also on modifications that were undertaken for the in vitro chick embryo system and the aggregate cultures of fetal rat brain cells. Today these tests cannot replace the existing in vivo developmental toxicity tests. They can, however, be used to screen chems. for further development or further testing. In addn., these in vitro tests provide valuable information on the mechanisms of developmental toxicity and help to understand the relevancy of findings for humans. In vitro systems, combined with selected in vivo testing and pharmacokinetic investigations in animals and humans, can thus provide essential information for human risk assessment.

95
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): 615-621.

Murine embryonal carcinoma (EC) cells, which resemble the undifferentiated cells of the epiblast in the blastocyst, were used to establish two in vitro assays for developmental toxicants. The target processes in the assays are the differentiation of EC cells into endodermal and mesodermal derivatives, respectively. 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 compounds that are teratogenic in vivo. The usefulness of the assays was studied with five pairs of xenobiotic compounds, with chemical 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 two is explicable in terms of mechanisms of action in vivo. Validation of the assays for classes of related compounds will determine their usefulness as a screen for each class, depending on the mechanism of action and physical properties. In this way in vitro assays will contribute to the refinement of testing, in addition to the more efficient use of laboratory animals.

96
Katoh M, Kimura R, Shoji R. IN VITRO DEVELOPMENTAL TOXICITY ASSAY SYSTEM SEARCH FOR EMBRYOGENESIS PROMOTERS EXISTING IN RAT SERUM IN WHOLE EMBRYO CULTURE. Thirty-Third Annual Meeting of the Japanese Teratology Society, Nagoya, Japan, July 21-23, 1993. Teratology 1993; 48(5):492.

No abstract.

97
Whittaker SG, Faustman EM. IN VITRO ASSAYS FOR DEVELOPMENTAL TOXICITY. Gad, S. C. (ED.). In Vitro Toxicology. IX+290P. Raven Press: New York, New York, USA. ISBN 0-88167-974-7. 1994;0(0):97-122.

No abstract.

98
Matsumoto H, Noda Y, Goto Y, Kishi J, Nonogaki T, Mori T. THE EFFECT OF HEAVY METAL IONS ON THE IN VITRO DEVELOPMENT OF MOUSE EMBRYOS: A COMPARISON OF THE DEVELOPMENTAL ABILITY BETWEEN HAM'S F-10 AND ALPHA-MEM. J Reprod Dev 1993;39(3):223-228.

To elucidate what kind of medium would be desirable for mammalian embryo culture, we evaluated the effect of heavy metal ions on mouse in vitro embryonic development. Pronuclear stage embryos recovered from ICR mice were cultured in Ham's F-10 and alpha-MEM and the developmental ability was compared between them. The major difference of these 2 media is that only Ham's F-10 contains heavy metal ions such as Zn2+, Fe2+ and Cu2+ , and hypoxanthine. When pronuclear stage embryos were cultured in alpha-MEM, the rates of embryos reaching the 4-cell, blastocyst and hatched blastocyst stages were 96.5%, 75.4% and 66.7%,

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

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

No abstract.

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

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

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

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

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

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

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

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

103
Smoak IW. DYSMORPHOGENIC EFFECTS AND TRANSPLACENTAL PASSAGE OF CHLOROBUTANOL IN MOUSE EMBRYOS DURING ORGANOGENESIS. Teratology 1993;47(5):436-7.

Chlorobutanol (1,1,1-trichloro-2-methyl-2-propanol) (CB) is a preservative in many intravenous and topical pharmaceutical preparations and is the active ingredient in certain local anesthetics and oral sedatives. Despite its widespread use, little is known regarding transplacental passage of CB or its effect on the developing embryo. In the present study, the method of whole-embryo culture (WEC) was used to expose neurulating (3-6 somite) mouse embryos to CB (3-30 ug/mL) in serum from rats dosed with 10-50 mg/kg CB by gastric feeding tube 2 hr prior to blood withdrawal. Serum levels of CB were determined by gas chromatography (GC). Embryos were examined after 24 hr in WEC for heart rate, malformations, somite number, and protein content. Embryos were malformed by exposure to CB in WEC at rates which increased with increasing CB concentrations: 1/16 (6%) at 3-5 ug/mL, 2/10 (20%) at 8-11 ug/mL, and 6/14 (43%) at 21-30 ug/mL. Defects included neural tube, optic, and cardiovascular abnormalities. Heart rate, somite, and protein values were also reduced by CB exposure. In addition, transplacental passage was determined by dosing pregnant mice with CB (8 and 80 mg/kg) by gastric feeding tube on gestational day 9.5 (plug = d 0.5) and assaying maternal serum and embryonic tissues 2 hr post-treatment for CB levels using GC. Pregnant mice treated with 8 mg/kg CB had serum concentrations of 0.5-1.2 ug/mL CB, and the drug was not detectable in embryonic tissues, whereas treatment with 80 mg/kg CB yielded 43 ug/mL in maternal serum and 2-10 ng CB in embryonic tissues. Thus, CB in serum from treated rats produces dysmorphogenesis in neurulating mouse embryos in vitro. In addition, CB appears to cross the placenta during organogenesis, when embryos are susceptible to its effects. The potential risk of CB to the developing embryo must be considered when using pharmaceutical preparations containing this compound.

105
Buttar HS, Smith S, Guest I, Varma DR. THE EFFECTS OF VALPROIC ACID AND CAPTOPRIL ON RAT EMBRYO DEVELOPMENT IN VITRO. Teratology 1993;47(5):402.

The primary focus of our ongoing studies is to determine if the rat embryos grown in culture can serve

as a model system to evaluate the teratogenic potential of pharmacologically unrelated drugs. Here we report results of 2 agents: the anticonvulsant, valproic acid (VPA), and the angiotensin converting enzyme inhibitor, captopril (CP), used in hypertensive therapy. Both VPA and CP are known to induce fetopathies in humans. On pregnancy day 10 (sperm positive = day 0), embryos were harvested from halothane anesthetized dams and cultured in rat serum for 48 hr in the absence or presence of increasing drug concentrations. At the end of 48 h, embryos were scored for morphological development according to the procedure of Brown and Fabro (Teratology 24:65, '81), and their total protein and DNA contents were determined. VPA (0.01-1.8 mM) caused concentration-dependent reduction in the morphological score of embryos and their DNA content. Also, VPA markedly increased visceral defects and inhibited yolk sac circulation, prevented axial rotation and neural tube closure, but there was no significant effect on embryo survival as judged by the heart beats. In contrast, CP (0.01-5 mM) neither exerted any apparent adverse effect on the morphological features nor changed the embryonic protein and DNA content even at the highest concentration. A complete lack of captopril embryotoxicity in rat conceptuses, whereas its alleged teratogenicity in humans, indicates that the whole-embryo culture may yield false negative data concerning such agents which may cause fetal anomalies by producing maternal-fetal hypotension. It is postulated that maternal hypotension would reduce utero-placental blood flow and consequently produce oligohydramnios and intrauterine growth retardation through curtailed nutrient and O2 supply to the developing fetus. Obviously many more agents from different chemical classes need to be tested in order to draw any definitive conclusion on the suitability of rodent embryo culture as a test system for potential human teratogens.

106
Tsuchiya T, Igarashi Y, Nakamura A. DEVELOPMENTAL TOXICITY OF URETHANE: IN VITRO TESTS USING VARIOUS EMBRYONIC CELLS AND LUNG ORGANS IN CULTURE. Teratol 1992;46(6):43B-44B.

Urethane is known to cause external malformations, lung anomalies, lung carcinoma etc., when administered to mice during the periods of embryonic organogenesis. We established methods of mouse embryonic lung organ- and cell-culture, and investigated the teratogenic or

carcinogenic action of urethane. Day 11 lung organs increased lung buds during culture in F12-fcs media. Day 17 lung cells formed many lumens during culture in modified F12-fcs media. In vivo/in vitro tests using the culture methods of micro mass cells of midbrain, limb bud and lung, or lung organs were also carried out. Inhibitory potencies on the differentiation of embryonic cells and organs by urethane were in the following order: lung cells greater than lung organ greater than midbrain cells greater than limb bud cells. While the inhibitory potencies on the differentiation of embryonic cells and organs by urethane using in vivo/in vitro tests were in the following order: lung cells greater than limb buds, midbrains greater than lung organs. From these results, urethane may be metabolically activated in both maternal and target tissues to induce anomalies and carcinoma in mice.

107
Reinhardt CA. NEURODEVELOPMENTAL TOXICITY IN-VITRO PRIMARY CELL CULTURE MODELS FOR SCREENING AND RISK ASSESSMENT. International Workshop on in Vitro Methods in Reproductive Toxicology, Ottawa, Ontario, Canada, May 19-20, 1992. Reprod Toxicol 1993;7(SUPPL. 1): 165-170.

No abstract.

EMBRYOTOXICITY
108
Heuer J, Bremer S, Pohl I, Spielmann H. DEVELOPMENT OF AN IN VITRO EMBRYOTOXICITY TEST USING MURINE EMBRYONIC STEM CELL CULTURES. Toxicol in Vitro 1993;7(4):551-6.

Mouse embryonic stem (ES) cell line D3 was used to establish conditions for a reproducible differentiation of ES cells in culture. ES cells can be maintained in an undifferentiated state by cultivation on a feeder layer of embryonic fibroblasts. ES cells form aggregates in suspension and can spontaneously differentiate into complex organized embryoid bodies (EBs), which in many respects resemble early postimplantation mouse embryos. Under appropriate culture conditions various cell and tissue types will develop in EBs: these include myocardial and skeletal muscle, nerve cells, chondrocytes and blood cells. Retinoic acid (RA) was used as an embryotoxic substance to test the application of ES cell cultures in in vitro

embryotoxicity testing. RA (1 .times. 10-8 M) induced an increase in skeletal muscle cell differentiation, which followed a characteristic pattern: day 10 is

characterized by the first appearance of mononucleated myoblasts; day 12 shows the fusion of myoblasts; on day 13, multinucleated myotubes can be detected, and on day 25 contractile myofibres are present in ES cell cultures. The development of blood islands with red cells enhanced by erythropoietin in EBs has encouraged the hope that, subsequently, more mature stages of erythroid, myeloid and lymphoid cell development could occur in vitro. These data provide further support for the use of ES cells in an in vitro assay for embryotoxicity testing.

109
Giavini E, Broccia ML, Menegola E, Prati M. COMPARATIVE IN VITRO STUDY OF THE EMBRYOTOXIC EFFECTS OF THREE GLYCOL ETHERS AND THEIR METABOLITES, THE ALKOXYACIDS. Toxicology In Vitro 1993;7(6):777-784.

Although the teratogenic potential of some glycol ethers and their active metabolites, the alkoxyacids, is well known, a comparative in vitro study of the embryotoxic potential of such compounds has not yet been conducted. The present study investigates the relationship between chemical structure and embryotoxicity of three glycol ethers (methoxy-, ethoxy- and butoxyethanol) and their corresponding alkoxyacids on the development of 9.5-day-old rat embryos cultured over 48 hr. The embryotoxic activity of the alkoxyacids was found to be higher than that of the corresponding ethers. Alkoxyacid embryotoxicity decreased with increasing length of the alkoxy chain, while the ether embryotoxicity increased with chain length. These data emphasize the need for a good knowledge of the metabolism of chemicals and the use of appropriate metabolic systems for reliable evaluation of results deriving from in vitro studies.

110
Saillenfait AM, Payan JP, Sabate JP, Langonne I, Fabry JP, Beydon D. SPECIFIC AMINO ACIDS MODULATE THE EMBRYOTOXICITY OF NICKEL CHLORIDE AND ITS TRANSFER TO THE RAT EMBRYO IN VITRO. Toxic Appl Pharm 1993;123(2):299-308.

To investigate the effects of amino acids on the

embryotoxicity and placental transfer of nickel chloride (NiCl2), Day 10 rat embryos were cultured in rat serum medium containing NiCl2 or 63NiCl2 (0.34 or 0.68 mM Ni), with or without L-histidine (2 mM), L-aspartic acid, glycine (2 or 8 mm), or L-cysteine (2 mm). After 26 hr, conceptuses were assessed for survival, growth and development, and malformations. The 63Ni contents of embryos and yolk sacs and the extent of 63Ni binding to the proteins of the culture medium were also determined. NiCl2 alone did not affect the embryonic development at 0.34 mm and caused growth retardation and brain and caudal abnormalities at 0.68 mm. Coincubation of L-histidine with 0.34 mm Ni increased Ni concentrations in embryonic tissues compared to 0.34 mm 63Ni alone, but did not elicit NiCl2 embryotoxicity. Coincubation of L-Cysteine with 0.34 mm Ni elicited growth retardation and brain abnormalities caused by NiCl2 and increased yolk sac concentrations of 63 Ni compared to 0.34 mm 63Ni alone. In contrast, coincubation of L-histidine, L-cysteine, or L-aspartic acid with 0.68 mm Ni reduced the growth retardation and the incidence and/or severity of brain defects caused by NiCl2 and decreased the concentrations of 63Ni in the yolk sacs, compared to 0.68 mm 63Ni alone. L-Histidine also reduced the percentage of NiCl2-elicited caudal defects. Coincubation with glycine did not affect the embryotoxic profile, nor the placental transfer of NiCl2. In the presence of L-histidine, L-cysteine, or L-aspartic acid, there was a shift of 63Ni binding from the high-molecular weight proteins of the culture medium to the low-molecular weight fraction. Thus, specific extracellular amino acids can modulate the embryotoxicity and placental transfer of NiCl2 in vitro. The pattern of this modulation is dependent on the concentration of NiCl2, as well as on the amino acid.

111
Smoak IW. EMBRYOTOXIC EFFECTS OF CHLOROBUTANOL IN CULTURED MOUSE EMBRYOS. Teratology 1993;47(3):203-8.

Chlorobutanol (CB) is a commonly used preservative which is added to numerous pharmaceutical preparations, and it is the active ingredient in certain oral sedatives and topical anesthetics. Chlorobutanol has demonstrated adverse effects in adult tissues, but CB has not been previously investigated for its effect on the developing whole embryo. The method of whole-embryo culture was used in this study to expose mouse embryos

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

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

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

methotrexate and cyclophosphamide produced an effect without prior exogenous activation as is necessary in other in vitro tests.

GASTROINTESTINAL TOXICITY

113

Ruby MV, Davis A, Link TE, Schoof R, Chaney RL, Freeman GB, Bergstrom P. DEVELOPMENT OF AN IN VITRO SCREENING TEST TO EVALUATE THE IN VIVO BIOACCESSIBILITY OF INGESTED MINE-WASTE LEAD. Environmental Science & Technology 1993;27(13):2870-2877.

A screening-level in vitro test was developed to evaluate the relative solubility of ingested lead (Ph) from different mine wastes in the gastrointestinal (GI) tract. The in vitro method, modeled after assay methods for available iron from food, used a laboratory digestion procedure designed to reproduce GI tract chemistry and function. The in vitro method was independently calibrated against a rabbit feeding study, demonstrating that only 1-6% of the total Ph in four mine-waste samples with disparate Ph mineralogy was bioaccessible. In vitro method development tests indicated that H+ concentration and Cl- complexation control dissolution of Pb-bearing minerals in the stomach and that both GI tract enzymes and organic acids are necessary to maintain Ph in the soluble form on entering the small intestine. The experimental results indicate that ingestion of Pb-bearing mine wastes results in limited Ph solubility and that the in vitro test provides. a screening-level estimate of the maximum available Ph from mine wastes.

114

Gad SC. GASTROINTESTINAL TOXICOLOGY IN VITRO TEST SYSTEMS. Gad, S. C. (ED.). In Vitro Toxicology. IX+290P. Raven Press: New York, New York, USA. ISBN 0-88167-974-7.; 0 (0). 1994. 231-237.

No asbstract.

115

Tang AS, Chikhale PJ, Shah PK, Borchardt RT. UTILIZATION OF A HUMAN INTESTINAL EPITHELIAL CELL CULTURE SYSTEM (CACO-2) FOR EVALUATING CYTOPROTECTIVE AGENTS. Pharm Res 1993; 10(11):1620-6.

Human intestinal epithelial cells (Caco-2) were

cultured as confluent monolayers on polycarbonate membranes in Transwells for investigating their applicability in evaluating the cytoprotective activity of sucralfate. The control expts. established a reproducible chem. method (using 0.5 mM indomethacin in Hanks' balanced salt soln.) for inducing damage to the Caco-2 cell monolayers. Damage was detd. by measuring changes in transepithelial elec. resistance (TEER). Twenty-day-old Caco-2 cell monolayers were significantly and reproducibly damaged (compared to buffer alone) ($P < 0.001$) by application of 0.5 mM indomethacin to the apical side for 1 h. While sucralfate, at a 0.5, 2, or 5 mg/mL concn. in the buffer, was shown not to reverse (treat) the damage caused by indomethacin in this cellular model, it was able to protect (prevent) the cells from indomethacin-induced damage ($P < 0.001$). The authors obsd. that indomethacin-induced damage to the Caco-2 cell monolayers greatly affected the paracellular pathway since the percentage transport of [3H]methoxyinulin was significantly elevated. In contrast, protection of the Caco-2 cells with 5 mg/mL sucralfate in the presence of the damaging agent resulted in transport of the paracellular marker similar to that in the control (HBSS-treated) cell monolayers. This direct cytoprotective effect was thus independent of vascular factors at neutral pH and was obsd. to be dose dependent (0.5 to 5 mg/mL) when sucralfate was applied to the cells in the presence of the damaging agent. These findings, which are consistent with those obsd. for sucralfate in vivo, demonstrate the feasibility of using Caco-2 cell monolayers as an in vitro cell culture system which may serve to identify and rapidly screen the cytoprotective activity of potential drugs and their pharmaceutical formulations.

GENOTOXICITY

116

Conti D, Catena C, Righi E. MICRONUCLEUS TEST IN THE STUDY OF THE TOXICITY OF CHEMICAL AGENTS. Arch Sci Lav 1993; 8(2):117-23.

The authors describe the results of a micronucleus assay on the in vitro CL-1/AF cells after treatment with caffeine, diethylstilbestrol, chloral hydrate, griseofulvin, and econazole. The authors conclude that this assay could be a useful test to indicate the genotoxicity of chem. and may be a better estn. of the genotoxicity of chem.

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

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

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

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

119
Warr TJ, Parry EM, Parry JM. A Comparison of Two In Vitro Mammalian Cell Cytogenetic Assays for the Detection of Mitotic Aneuploidy Using 10 Known or Suspected Aneugens. Mutat Res 1993;287(1):29-46.

This report concerns the study of ten chemicals examined with the major aim of developing a database on the chemical induction of aneuploidy in order to evaluate different test systems. The results obtained from two mammalian cytogenetic assays, the cell division aberration (CDA) assay and the chromosome enumeration (CE) assay, were reported for colchicine, vinblastine, chloral-hydrate, thiabendazole, hydroquinone, thimerosol, cadmium-chloride, econazole-nitrate, pyrimethamine and diazepam. Two Chinese-hamster cell lines of pulmonary origin, LUC2 and Don.Wg.3H, were employed but only the LUC2 was used for the chromosome enumeration tests. Of the ten test compounds, six induced both aneuploidy and tetraploidy in the LUC2 cultures: colchicine, vinblastine, chloral-hydrate, thiabendazole, pyrimethamine, and diazepam. Treatment with econazole-nitrate resulted in aneuploid progeny and thimerosol induced tetraploidy. A substantial degree of correlation was noted between the CDA and the CE assays in the LUC2 cell line. Chemicals which induced aneuploidy also induced appropriate divisional aberrations during the previous mitosis. Whenever tetraploidy was induced, the compound also induced mitotic arrest and disrupted spindle integrity. The authors suggest that the CDA assay appears suitable for use in routine screening of aneugens. They caution that the choice of cell line is critical.

120
Brusick DJ,Ashby J,de Serres FJ, Lohman PHM, Matsushima T. METHOD FOR COMBINING AND COMPARING SHORT-TERM GENOTOXICITY TEST DATA: PREFACE. A REPORT FROM ICPEMC COMMITTEE 1. Department of Energy, Washington, DC. Govt Reports Announcements & Index (GRA&I), Issue 24, 1993.

Short-term testing methods, encompassing a wide variety of species and genetic mechanisms, have been developed to detect genetic effects produced by chemicals. The use of multiplicity of tests has created a difficult and controversial challenge in the interpretation of mixed test results. The driving principle of this report and the International Commission for Protection against Environmental Mutagens and Carcinogens (ICPEMC)

Committee 1 was to combine the major parameters of testing (dose, metabolic activation, sign of response, and the replication of test data) into a single score which could be pooled by entry, by test (e.g., Salmonella reverse mutation), by test class (e.g., bacterial mutation), and by family (in vitro or in vivo), into a composite score for a chemical. The system is designed: (i) to cope with redundant data, disagreement, and sporadically filled matrices; (ii) to supply statistical properties by chemical and by test; and (iii) to have features of self-learning to improve predictive performance and internal consistency for any one of several types of genetic hazard. Journal article. Pub. in Mutation Research, v266 n1 p7-25 Mar 92. See also PB89-109680. Prepared in cooperation with Leiden Rijksuniversiteit (Netherlands), Lawrence Livermore National Lab., CA., and Hazleton Labs. America, Inc., Vienna, VA. Sponsored by Department of Energy, Washington, DC.

121
Vogel R. IN VITRO APPROACH TO FERTILITY RESEARCH: GENOTOXICITY TESTS ON PRIMORDIAL GERM CELLS AND EMBRYONIC STEM CELLS. Reprod Toxicol 1993;7(Suppl 1):69-73. (27 REFS)

The potential uses of primordial germ cells and embryonic stem cell cultures for assessing female reproductive system toxicity were discussed. New guidelines of the European Economic Community and the Organization for Economic Cooperation and Development have mandated in-vitro screening tests for potential reproductive toxicants. In-vitro tests were recommended because the large numbers of chemicals that must be tested would consume too many animals and be too expensive if in-vivo assays were used. Appropriate in-vitro systems for investigating toxicant effects on female fertility do not exist. It was suggested that pluripotent embryonic stem cells could be used to develop an in-vitro model for screening chemicals for female reproductive toxicity. Techniques for preparing and maintaining primordial germ cells and embryonic stem cells derived from NMRI-mouse blastocysts in culture were described. Embryonic stem cell cultures were found to be easily established and maintained and to have cellular and biochemical properties similar to primordial germ cells. Because of this, the author suggests that embryonic stem cell cultures can be used to assess the effects of toxicants on the female reproductive system.

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

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

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

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

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

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

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

potential for DGBE.

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

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

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

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

No abstract.

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

No abstract.

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

No abstract.

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

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

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

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

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

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

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

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

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

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

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

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

field suggests that rigid test protocols and a restrictive test battery would be inadequate.

IMMUNOTOXICITY

134
Fautz R, Miltenburger HG. IMMUNOTOXICITY SCREENING IN VITRO USING AN ECONOMICAL MULTIPLE ENDPOINT APPROACH. Toxicol in Vitro 1993;7(4):305-10.

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

135
Kashima R, Okada J, Ikeda Y, Yoshizuka N. CHALLENGE ASSAY IN VITRO USING LYMPHOCYTE BLASTOGENESIS FOR THE CONTACT HYPERSENSITIVITY ASSAY. Food and Chem Toxicol 1993; 31(10):759-766. (18 REFS)

To confirm positivity in routine guinea pig studies, contact allergenicity was investigated by a challenge assay in vitro using a co-culture of autologous lymphocytes passed through a nylon-wool column and

antigen- presenting cells (APCs) modified with or without antigen. Proliferation of the lymphocytes primed with ovalbumin and/or 2,4-dinitrochlorobenzene was antigen specific and dependent on the presence of APCs (peripheral blood monocytes, splenic macrophages and macrophages induced by liquid paraffin). For another nine haptens, primed lymphocytes proliferated significantly more than control lymphocytes; the stimulation index (SI; ratio between (3H)methylthymidine ((3H)TdR) incorporation of lymphocytes with antigen-modified APCs and (3H)TdR incorporation of lymphocytes with APCs not modified by antigen) was 1.6-4.8 in sensitized animals whereas it was about 1.0 in control animals. Sodium dodecyl sulfate did not cause lymphocyte proliferation. The SI value in vitro was correlated with both the positive rate in vivo (r = 0.736) and the mean response score in vivo (r = 0.645). Thus, it was possible to confirm that positivity in routine experiments was a true sign of allergy. A combination of this assay and short-term animal studies would provide an efficient assessment of the allergic potential of chemicals.

136
Oliver JD, Hauck PR. IN VITRO CHARACTERIZATION AND POTENCY ASSESSMENT OF AQUEOUS ALUMINUM PRECIPITATED GRASS POLLEN EXTRACTS. Fiftieth Annual Meeting of the American Academy of Allergy and Immunology, Anaheim, California, USA, March 4-9, 1994. J Allergy and Clinic Immunol 1994;93(1 PART 2):191.

No abstract.

137
Moulin C, Peguet-Navarro J, Courtellemont P, Redziniak G, Schmitt D. IN VITRO PRIMARY SENSITIZATION AND RESTIMULATION OF HAPTEN-SPECIFIC T CELLS BY FRESH AND CULTURED HUMAN EPIDERMAL LANGERHANS' CELLS. Immunology 1993; 80(3):373-379.

We examined the capacity of human Langerhans' cells (LC) to sensitize autologous T cells to the trinitrophenyl hapten (TNP) in vitro. Two-day cultured Langerhans' cells, but not freshly prepared Langerhans' cells, can induce in vitro primary proliferative reactions to the TNP hapten. Using a CD45RA+ naive T-cell subset, similar results were found, therefore making the possibility of a previous in vivo T-cell contact with the hapten unlikely. The primary in vitro

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

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

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

to be pro-inflammatory, were monitored in the MDA by the acute inflammation marker. Skin irritation scores of known preparations correlated well with those of expressed acute inflammation markers in the MDA (rs = 0.714), but no clear relationship was detectable in the LTT. In contrast one of the CTFA samples tested blind revealed a strong response in both tests. The roll-on antiperspirant stimulated T-cell proliferation and induce a strong expression of the acute inflammation marker on macrophages. Based on these findings further studies are in progress to evaluate the usefulness of these in vitro tests for predicting dermal irritation.

139
De Silva O, Perez MJ, Pineau N, Rougier A, Dossou KG. LOCAL LYMPH NODE ASSAY: STUDY OF THE IN VITRO PROLIFERATION AND CONTROL OF THE SPECIFICITY OF THE RESPONSE BY FACSCAN ANALYSIS. Toxic In Vitro 1993;7(4):299-303.

The murine local lymph node assay (LLNA) has been proposed as a screening procedure to identify contact allergens (Kimber, Hilton and Weisenberger, Contact Dermatitis 1989, 21, 215; Kimber and Weisenberger, Archives of Toxicology 1989, 63, 274). In some cases irritants have given rise to proliferative responses and it is of interest to investigate whether these responses differ in the type of cells involved. We have studied the proliferative response in vitro to topically applied sodium lauryl sulphate (SLS) and 2,4-dinitrochlorobenzene (DNCB). The test chemical or vehicle alone was applied for 3 consecutive days to the dorsum of both ears of Balb/c strain mice at three different concentrations, the highest concentration being the maximum non-irritating concentration (MNIC). Cell cultures were made 72 hr after the final exposure: draining auricular lymph nodes were excised and a suspension of lymph node cells (LNC) was prepared. Cellularity (total number of LNC/animal) and proliferative activity were assessed, proliferation was measured by culture of LNC for 24 hr with (3H)thymidine. LNC were also studied by FACScan analysis: cells were incubated with fluorescein isothiocyanate (FITC)-labelled monoclonal antibodies against the T-cell marker CD3 and the activated T-cell marker CD25 (IL2 receptor). In the case of DNCB dose-response curves were obtained for both cellularity and proliferative response in comparison with the controls: there was a strong increase in both

parameters for the MNIC.SLS, a non-sensitizing skin irritant, induced a much lower response, slightly increased at the MNIC in comparison with the controls. By FACScan analysis we measured the rates of CD3- and CD25-positive cells in the LNC. No significant difference was obtained for SLS in comparison with the controls. In the case of DNCB, there was a significant increase in CD3-positive cells and a large increase in CD25-positive cells in comparison with the controls and SLS. These parameters could be of great interest to help distinguish between contact sensitizers and irritants. We are presently investigating other irritants and sensitizers.

140
Lang DS, Meier KL, Luster MI. COMPARATIVE EFFECTS OF IMMUNOTOXIC CHEMICALS ON IN VITRO PROLIFERATIVE RESPONSES OF HUMAN AND RODENT LYMPHOCYTES. Fundam Appl Toxicol 1993; 21(4):535-45.

In order to determine the comparability of human and rodent in vitro systems, the direct effects of various therapeutic or environmental chemicals on proliferative responses of lymphocytes of mouse, rat, and human origins were examined and analyzed by a detailed statistical approach. Four compounds of diverse structure and mechanism of action which are known to impair lymphocyte transformation, such as hydroquinone, T-2 toxin, lead nitrate, as well as the widely used immunosuppressive drug cyclosporin A, were chosen as model test substances. T cells were stimulated by phytohaemagglutinin as well as monoclonal antibodies directed at the T cell receptor/CD3 complex, while B cells were activated by the T-independent mitogens, including Staphylococcus aureus cells, Escherichia coli lipopolysaccharide, and Salmonella typhimurium mitogen with specificity for human, mouse, and rat lymphocytes, respectively. In almost all cases the chemicals altered lymphoproliferative responses in a concentration-related manner in all three species. In general, overall similarities in the relative sensitivity of lymphoblastogenesis were obtained when the human dose-response curves were compared to the rodent response curves. Frequent, statistically significant species-dependent discrepancies of the overall response curves between mice and rats were observed. Large, statistically significant differences were observed for inorganic lead, revealing obvious divergences of the effect patterns in all cases, across all species. In this case, rodent species, especially the rat, were

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

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

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

METABOLISM/XENOBIOTICS
142
Zhang XX, Chakrabarti S, Malick AM, Richer CL. EFFECTS OF DIFFERENT STYRENE METABOLITES ON

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

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

143
Rogiers V, Vercruysse A. RAT HEPATOCYTE CULTURES AND CO-CULTURES IN BIOTRANSFORMATION STUDIES OF XENOBIOTICS. Toxicol 1993;82(1-3):193-208.

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

cell-cell interactions and factors affecting replication. For the maintenance of liver-specific functions, it seems of utmost importance that the tridimensional shape of the hepatocytes is kept. Usually, phase II enzymatic activity is better kept than that of phase I. The cytochrome P450 dependent monoxygenases, in particular, are easily lost. Interesting is the observation that co-cultures of rat hepatocytes with rat liver epithelial cells exhibit higher and much better preserved phase I and phase II biotransformation than monocultures. Clearly, further research is needed to improve this promising in vitro model.

144
Doehmer J. V79 CHINESE HAMSTER CELLS GENETICALLY ENGINEERED FOR CYTOCHROME P450 AND THEIR USE IN MUTAGENICITY AND METABOLISM STUDIES. Toxicology 1993;82(1-3):105-118.

V79 Chinese hamster cells are being genetically engineered for stable expression of cytochromes P450 using a SV40 early promoter containing plasmid as the eukaryotic expression vector. V79 cells lack endogenous cytochrome P450 activity. Therefore, genetically engineered V79 cell lines are defined for the cytochrome P450 isoform acquired upon cDNA mediated gene transfer. V79 cells have a longstanding tradition as indicator cells for various biological endpoints, e.g. mutation, chromosomal aberration, cytotoxicity. The genetically engineered V79 cells combine these useful biological endpoints with metabolic competence. In this sense, V79 cell lines genetically engineered for stable expression of cytochromes P450 present newly developed tools for studying and understanding metabolism related problems in toxicology and pharmacology.

145
Degen GH. PROSTAGLANDIN-H SYNTHASE CONTAINING CELL LINES AS TOOLS FOR STUDYING METABOLISM AND TOXICITY OF XENOBIOTICS. Toxicology 1993;82(1-3):243-56. (REFS: 54)

Prostaglandin-H synthase (PHS) can oxidize many xenobiotics and carcinogens (chemicals) in vitro and has been suggested to serve as an alternative metabolic activation enzyme, particularly in tissues low in monooxygenase activity. This article briefly describes

types of PHS-catalyzed xenobiotic oxidations and discusses its determinants in cells. Methods employed for studying the involvement of PHS in the bioactivation of chemicals are reviewed with special emphasis on a cell culture system derived from ram seminal vesicles which has been used in studies on the metabolism and the genotoxicity of diethylstilbestrol.

MUTAGENICITY
146
Mueller K, Kasper P, Mueller L. AN ASSESSMENT OF THE IN VITRO HEPATOCYTE MICRONUCLEUS ASSAY. Mutat Res 1993;292(3):213-24.

The in vitro hepatocyte micronucleus assay was tested for its practicability and its usefulness in detecting mutagens. The assay protocol developed by Alati et al. (1989) was shown to give reproducible levels of proliferating hepatocytes and the formation of micronuclei could be readily assessed by fluorescence microscopy. Epidermal growth factor and insulin were

used as mitogens, yielding mitotic indexes of 2.4 .+-. 0.74% after 72 h of culture. The high no. of 8.0 .+-. 3.33 micronucleated hepatocytes in control cultures at that time, typically for in vitro stimulated hepatocytes, is probably due to disordered mitoses frequently leading to chromosome loss. The direct acting mutagen N-methyl-N'-nitro-N-nitrosoguanidine and the clastogens cyclophosphamide and retrorsine, which require metabolic activation, induced dose dependent increases in the frequencies of micronucleated hepatocytes. The carcinogen 2-AAF also yielded significantly enhanced rates of micronuclei. The non-mutagen KCl as well as the peroxisome proliferator clofibrate, which is considered to be a non-genotoxic hepatocarcinogen, yielded consistently neg. results. Problems occurred when chems. exerting strong cytotoxic effects yielded consistently neg. results. Problems occurred when chems. exerting strong cytotoxic effects were tested in this assay. The mutagen and hepatocarcinogen aflatoxin B1 did not enhance the number of micronucleated hepatocytes. Rather a redn. of micronuclei and of mitoses was obsd. at AFB1 concentrations considered pos. in other genotoxicity assays. Hepatocyte proliferation seems to be highly susceptible to the cytotoxic action of chems. A decrease in the proliferating activity of hepatocytes can obviously prevent the detection of mutagenic effects. Further studies on the in vitro hepatocyte

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

147
Blakey DH, Maus KL, Bell R, Bayley J, Douglas GR, Nestmann ER. MUTAGENIC ACTIVITY OF 3 INDUSTRIAL CHEMICALS IN A BATTERY OF IN VITRO AND IN VIVO TESTS. Mutat Res 1994; 320(4):273-83.

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

148
Bean CL, Galloway SM. EVALUATION OF THE NEED FOR A LATE HARVEST TIME IN THE ASSAY FOR CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY CELLS. Mutat Res 1993; 292(1):3-16.

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

aberrations at both time points. Dimethylnitrosamine (DMN) induced more aberrations at 44 hours compared with 20 hours. The aberrations that were induced primarily at 20 hours by the first group of substances and those that were induced equally at both time points by the second group were mostly seen in cells in the first metaphase (M1). The aberrations induced by the first group, however, were observed in all three metaphases at 44 hours while those induced by the second group were still concentrated in M1 cells. The aberrations induced by DMN were seen in M1 and M1 second division cells at 20 hours and second and third division cells at 44 hours. Chromatid deletions (TD) and exchanges (TE) were seen at 20 hours by the first group of chemicals with the exception of BP. Group two chemicals and DMN induced TD, TE, and isochromatid deletions. The authors conclude that a 20 hour harvest time was useful for observing chromosomal aberrations induced by the ten chemicals tested.

149
Hamasaki T, Sato T, Nagase H, Kito H. THE MUTAGENICITY OF ORGANOTIN COMPOUNDS AS ENVIRONMENTAL POLLUTANTS. Mutat Res 1993;300(3/4):265-271. (31 REFS)

The mutagenicity of organotins and stannic-chloride was investigated. Twelve organotin compounds and stannic-chloride were tested for mutagenicity in a modified Ames/Salmonella assay using strains (TA-100) and (TA-98). The assay was modified by washing the tester strains with phosphate buffer and removing each tested chemical before counting the number of histidine positive (His+) revertants, thus avoiding problems caused by the bactericidal activity of the organotins. The number of surviving (TA-98) and (TA-100) colonies was determined in each assay. Test concentrations ranged from 0.1 to 100 micrograms per tube. Mono-n-butyltin-oxide (MBTO), n-butyltin-trichloride (BTTC), di-n-butyltin-dichloride (DBTDC), tri-n-butyltin-chloride (TBTC), bis(tri-n-butyltin)-oxide (BTBTO), and dimethyltin-dichloride (DMTDC) were mutagenic in strain (TA-100). When the number of His+ revertants was corrected by the number of surviving colonies, BTBTO was found to be the most mutagenic. The other organotins and stannic-chloride were inactive in strain (TA-100). When tested in strain (TA-98), DBTDC was the only mutagenic compound found. The authors note that the identified mutagenic compounds MBTO, BTTC, DBTDC, TBTC, BTBTO, and DMTDC are reported environmental

pollutants. The modified Ames/Salmonella assay is useful for detecting mutagenic activity in organotins and other compounds that have significant antibacterial activity.

150
Natarajan AT. AN OVERVIEW OF THE RESULTS OF TESTING OF KNOWN OR SUSPECTED ANEUGENS USING MAMMALIAN CELLS IN VITRO. Mutat Res 1993;287(1):113-118. (16 REFS)

In-vitro tests used for testing known or suspected aneugens were described and the results obtained for each of the substances tested were presented. The endpoints included micronuclei, kinetochore positive micronuclei in binucleated cells, changes in the number of chromosomes or aberrations of mitosis and division. Target cells were human lymphocytes, human diploid fibroblasts and Chinese-hamster transformed cells. One disadvantage noted with the human cells was the interindividual variation in base line frequency of micronuclei as well as interindividual variation in response to treatment. Variations were also noted in the response to the two Chinese-hamster cell lines. Diazepam was positive in Cl1 cells (Chinese-hamster established cell line) and negative in LUC2 cells (Chinese-hamster lung fibroblasts). In human cells the diazepam, hydroquinone, econazole and cadmium- chloride were positive for inducing kinetochore containing micronuclei, but were negative in Chinese-hamster LUC2 cells. Cadmium-chloride was positive in CHEL cells (Chinese-hamster embryonic fibroblasts) and negative in LUC2 cells. Pyrimethamine was negative in CHEL cells and positive in LUC2 cells. Results obtained with CHEL cells compared well with the results obtained using human lymphocytes.

151
Lynch AM, Parry JM. THE CYTOCHALASIN-B MICRONUCLEUS/KINETOCHORE ASSAY IN VITRO: STUDIES WITH 10 SUSPECTED ANEUGENS. Mutat Res 1993;287(1):71-86. (83 REFS)

The application of an in-vitro micronucleus (MN) assay using low passage Chinese-hamster lung fibroblasts for

the detection of aneugenic chemicals was investigated. Colchicine, vinblastine, thiabendazole, chloral-hydrate, thimerosal, diazepam, pyrimetha-mine, hydroquinone, cadmium-chloride, and econazole- nitrate

were the test chemicals studied. In low passage Chinese-hamster lung fibroblasts (LUC2), colchicine, vinblastine, thiabendazole, chloral-hydrate, diazepam, pyrimethamine, and cadmium-chloride induced significant increases in MN frequency. Colchicine, vinblastine, thiabendazole, and chloral-hydrate increased the levels of micronuclei which were positive for kinetochore antibody labeling and hence chromosome loss. Diazepam, pyrimethamine, and hydroquinone, and the positive control mitomycin-C increased the levels of MN negative for kinetochore antibody labeling. Thimerosal gave only equivocal results and econazole-nitrate was negative. The authors conclude that the cytochalasin-B MN/kinetochore assay is cost effective, simple and rapid as an alternative to classical cytogenetic assays for detecting chemically induced aneuploidy.

152
Sbrana I, Di Sibio A, Lomi A, Scarcelli V. C-MITOSIS AND NUMERICAL CHROMOSOME ABERRATION ANALYSES IN HUMAN LYMPHOCYTES: 10 KNOWN OR SUSPECTED SPINDLE POISONS. Mutat Res 1993;287(1):57-70. (23 REFS)

Human lymphocyte cultures were exposed to cadmium-chloride, chloral-hydrate, colchicine, diazepam, econazole, hydroquinone, pyrimethamine, thiabendazole, thimerosol and vinblastine in an effort to validate suitable assays for chemically induced genomic mutations, chromosomal aberrations and spindle effects. Treatment was carried on for 48 to 72 hours after which chromosome number assays were determined. Spindle effects were analyzed in cultures treated 5 hours prior to fixation. Colchicine and vinblastine produced dose related numerical chromosomal aberrations and were the only two chemicals which also produced C-mitotic effects over a wide range of dose levels. Chloral-hydrate, cadmium-chloride, and thimerosol caused hyperdiploidy without a dose effect relationship. Spindle functions were affected by chloral-hydrate and thimerosol, while only a weak spindle effect was produced by cadmium-chloride. Hydroquinone, thiabendazole, and thimerosol each induced tetraploid and/or endoreduplicated cells without a dose effect relationship. Only hypodiploidy was induced by diazepam and econazole. No induction of numerical chromosomal aberrations was noted following treatment with pyrimethamine.

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Natarajan AT, Duivenvoorden WCM, Meijers M, Zwanenburg TSB. INDUCTION OF MITOTIC ANEUPLOIDY USING CHINESE HAMSTER PRIMARY EMBRYONIC CELLS. TEST RESULTS OF 10 CHEMICALS. Mutat Res 1993;287(1):47-56. (17 REFS)

The ability of ten chemical to induce mitotic aneuploidy was studied in a test system using Chinese-hamster primary embryonic cells. Diploid Chinese-hamster embryonic fibroblasts were grown on coverslips and were exposed to the chemicals by replacing the culture medium with fresh medium containing the test substance. Depression of the mitotic index and reduction of cells in second and third divisions following preliminary toxicity tests were used as the measurement tools. Five chemicals, cadmium-chloride, chloral-hydrate, diazepam, pyrimethamine, and thiabendazole were tested for induction of aneuploidy. The other five chemicals, colchicine, econazole-nitrate, hydroquinone, vincristine and thimerosal, were subjected to testing in an industrial setting including prescreening for cytotoxicity using the mitotic index and cell growth as parameters, selection of the top concentration in the main experiments, inclusion of a positive control, and performance of an independent repeat. All chemicals, except pyrimethamine, were clearly positive. The authors conclude that the in-vitro test described using primary embryonic fibroblasts is very suitable for routine testing to detect aneugenic chemicals.

154
Parry JM. AN EVALUATION OF THE USE OF IN VITRO TUBULIN POLYMERISATION, FUNGAL AND WHEAT ASSAYS TO DETECT THE ACTIVITY OF POTENTIAL CHEMICAL ANEUGENS. Mutat Res 1993;287(1):23-28. (11 REFS)

Ten test chemicals were studied by seven collaborating laboratories to evaluate the ability of the chemicals to produce aneuploidy or aneuploidy related endpoints in specific assays using in-vitro tubulin polymerization, fungi and wheat. The responses of the assays to these ten chemicals differed significantly. No response was obtained on fungal assays to colchicine and vinblastine. Only three chemicals were positive in all the fungal test systems used, chloral-hydrate, thimerosol, and thiabendazole. Unambiguous positive results were obtained with the in-vitro tubulin polymerization assays for colchicine, thimerosol and vinblastine-sulfate. A positive response was obtained with eight of the ten chemicals using the hexaploid

wheat assay. These eight included colchicine, econazole, thimerosol, pyrimethamine, thiabendazole, cadmium-chloride, vinblastine and diazepam. The author concludes that none of the systems studied was able to detect the potential aneugenic activity of each of the ten chemicals. The fungal assays appeared severely limited with their inability to detect potent mammalian spindle poisons such as vinblastine and colchicine.

155
Oda Y, Yamazaki H, Watanabe M, Nohmi T, Shimada T. HIGHLY SENSITIVE UMU TEST SYSTEM FOR THE DETECTION OF MUTAGENIC NITROARENES IN SALMONELLA TYPHIMURIUM NM3009 HAVING HIGH O-ACETYLTRANSFERASE AND NITROREDUCTASE ACTIVITIES. Environment Molec Mutagen 1993;21(4):357-364. (40 REFS)

A method to detect the genotoxic activities of nitroarenes using a new tester strain of Salmonella-typhimurium, strain NM3009, having high O-acetyltransferase (O-AT) and nitroreductase (NR) activities was described. Strain NM3009 was constructed by subcloning the O-AT and NR genes into a plasmid vector and introducing the vector into the parent test strain, (TA-1535)/pSK1002 which had an umuC'/lacZ fusion gene. The induction of umuC gene expression was monitored by assaying the level of beta-galactosidase activity produced by the fusion gene. The sensitivity of strain NM3009 to nitroarenes was compared with those of the parent strain, an NR overexpressing strain, and NR deficient strain, an O-AT over expressing strain, and an O-AT defective strain. All of the strains tested demonstrated spontaneous expression of the umuC gene in the absence of genotoxins. Sensitivity to nitroarene compounds was enhanced in strain NM3009 up to 720 times the level seen in the parent strain and the sensitivity of strain NM2009 to these compounds was increased up to 240 times. Strain NM1000 did not show any reactivity to the test compounds and strain NM2000 demonstrated some sensitivity to all but one of the chemicals. The authors conclude that the new tester strain NM3009 is useful for detecting genotoxic activities and chemicals which require NR and/or O-AT activity for activation.

156
McKelvey-Martin VJ, Green MHL, Schmezer P, Pool-Zobel BL, De Meo MP, Collins A. THE SINGLE CELL GEL ELECTROPHORESIS ASSAY (COMET ASSAY): A EUROPEAN REVIEW. Mutat Res 1993; 288(1):47-63. (41 REFS)

This review discussed the development of the single cell gel electrophoresis (SCGE) assay, existing protocols for the detection and analysis of comments, the relevant underlying principles determining the behavior of DNA, and the potential applications of the technique. The SCGE assay was developed in 1984 to measure DNA breakage in mammalian cells. Specific topics covered in the review included the development of the method, the SCGE assay pro tocol, optional variations to the described protocol, treatment of cells for analysis in the SCGE assay, agents tested in the SCGE assay, analysis of DNA breakage in the SCGE assay, underlying principles determining the behavior of DNA in the SCGE assay, and potential applications. The SCGE assay has been used in both in-vitro and in-vivo studies to assess DNA damage and repair induced by various agents in a variety of mammalian cells. Widespread applications in DNA damage and repair studies, biomonitoring, genetic toxicology and analysis of irradiated food have been found.

157
van Loon AAWM, Sonneveld E, Hoogerbrugge J, van der Schans GP, Grootegoed JA, Lohman PHM, Baan RA. INDUCTION AND REPAIR OF DNA SINGLE-STRAND BREAKS AND DNA BASE DAMAGE AT DIFFERENT CELLULAR STAGES OF SPERMATOGENESIS OF THE HAMSTER UPON IN VITRO EXPOSURE TO IONIZING RADIATION. Mutat Res 1993;294(2):139-148. (33 REFS)

The in-vitro induction and repair of two classes of DNA damage, single strand breaks (SSB) and base damage (BD), were investigated in spermatocytes, round and elongated spermatids, and bone marrow cells isolated from Syrian-golden-hamsters. Cells were treated with up to 8 gray gamma radiation. Quantification was achieved of radiation induced DNA damage in the mammalian cells without the use of radioactively labeled cells by alkaline elution. Substantial differences were noted in lesion induction and repair capacity between different stages of germ cell development. Spermatocytes, round spermatids and bone marrow cells had normal repair of the SSB when compared with the repair reported for cultured rodent cells and human lymphocytes. The elongated spermatids showed hardly any SSB repair. The initial rate of repair of BD in spermatocytes and bone marrow cells was in the same range as that for SSB, but only 60 to 70% of the initial BD was repaired within 1 hour, while after that period no SSB were detectable.

Round spermatids hardly repaired any BD within the first hour after irradiation, but after 7 hours only a few BD could be detected. The repair of BD in elongated spermatids could not be measured due to a high background level of this type of damage. These findings indicated that there was a stage dependent induction of DNA damage and a gradual loss of the capacity to repair SSB and BD during spermatogenesis. The authors suggest that this information may be relevant in the context of studies on genetic risk. Application of the assays may contribute to a greater understanding of the role of DNA damage in mutagenesis.

158
Muller K, Kasper P, Muller L. AN ASSESSMENT OF THE IN VITRO HEPATOCYTE MICRONUCLEUS ASSAY. Mutat Res 1993;292(3):213-24.

The in vitro hepatocyte micronucleus assay was tested for its practicability and its usefulness in detecting mutagens. The assay protocol developed by Alati et al. (1989) was shown to give reproducible levels of proliferating hepatocytes and the formation of micronuclei could be readily assessed by fluorescence microscopy. Epidermal growth factor and insulin were used as mitogens, yielding mitotic indices of 2.4 +/- 0.74% after 72 h of culture. The high number of 8.0 +/- 3.33% micronucleated hepatocytes in control cultures at that time, typically for in vitro stimulated hepatocytes, is probably due to disordered mitoses frequently leading to chromosome loss. The direct acting mutagen N-methyl-N'-nitro-N-nitrosoguanidine and the clastogens cyclophosphamide and retrorsine, which require metabolic activation, induced dose dependent increases in the frequencies of micronucleated hepatocytes. The carcinogen 2-AAF also yielded significantly enhanced rates of micronuclei. The non-mutagen KCl as well as the peroxisome proliferator clofibrate, which is considered to be a non-genotoxic hepatocarcinogen, yielded consistently negative results. Problems occurred when chemicals exerting strong cytotoxic effects were tested in this assay. The mutagen and hepatocarcinogen aflatoxin B1 did not enhance the number of micronucleated hepatocytes. Rather a reduction of micronuclei and of mitoses was observed at AFB1 concentrations considered positive in other genotoxicity assays. Hepatocyte proliferation seems to be highly susceptible to the cytotoxic action of chemicals. A decrease in the proliferating activity of hepatocytes can obviously

prevent the detection of mutagenic effects. Further studies on the in vitro hepatocyte micronucleus assay are necessary to clarify its role in mutagenicity testing.

159
Bouquet M, Selva J, Auroux M. CRYOPRESERVATION OF MOUSE OOCYTES: MUTAGENIC EFFECTS IN THE EMBRYO? Biol Reprod 1993;49(4):764-9.

We have shown in previous studies that the complete cycle of cryopreservation and prefreezing manipulations increases the degeneration and decreases the fecundability of mouse oocytes. The present study confirms these results. Moreover, we show that the increase of polyploidy previously observed in one-cell zygotes derived from frozen-thawed oocytes persists during the early stages of embryonic development. Furthermore, embryos obtained from frozen oocytes or oocytes exposed to prefreezing manipulations show an increase in the frequency of sister chromatid exchanges. Since the estimation of sister chromatid exchange is a sensitive test of mutagenicity, this suggests that the complete cycle of cryopreservation might alter the oocyte and, more particularly, induce DNA damage.

160
Muller K, Kasper P, Muller L. AN ASSESSMENT OF THE IN VITRO HEPATOCYTE MICRONUCLEUS ASSAY. Mutat Res 1993;292(3):213-24.

The in vitro hepatocyte micronucleus assay was tested for its practicability and its usefulness in detecting mutagens. The assay protocol developed by Alati et al. (1989) was shown to give reproducible levels of proliferating hepatocytes and the formation of micronuclei could be readily assessed by fluorescence microscopy. Epidermal growth factor and insulin were used as mitogens, yielding mitotic indices of 2.4 +/- 0.74% after 72 h of culture. The high number of 8.0 +/- 3.33% micronucleated hepatocytes in control cultures at that time, typically for in vitro stimulated hepatocytes, is probably due to disordered mitoses frequently leading to chromosome loss. The direct acting mutagen N-methyl-N'-nitro-N-nitrosoguanidine and the clastogens cyclophosphamide and retrorsine, which require metabolic activation, induced dose dependent increases in the frequencies of micronucleated

hepatocytes. The carcinogen 2-AAF also yielded significantly enhanced rates of micronuclei. The non-mutagen KCl as well as the peroxisome proliferator clofibrate, which is considered to be a non-genotoxic hepatocarcinogen, yielded consistently negative results. Problems occurred when chemicals exerting strong cytotoxic effects were tested in this assay. The mutagen and hepatocarcinogen aflatoxin B1 did not enhance the number of micronucleated hepatocytes. Rather a reduction of micronuclei and of mitoses was observed at AFB1 concentrations considered positive in other genotoxicity assays. Hepatocyte proliferation seems to be highly susceptible to the cytotoxic action of chemicals. A decrease in the proliferating activity of hepatocytes can obviously prevent the detection of mutagenic effects. Further studies on the in vitro hepatocyte micronucleus assay are necessary to clarify its role in mutagenicity testing.

NEPHROTOXICITY

161
Klein J, Koren G, Mcleod SM. COMPARISON OF METHODS FOR PREDICTION OF NEPHROTOXICITY DURING DEVELOPMENT. Dev Pharmacol Ther 1993;19(2-3):80-89.

Drugs with nephrotoxic potential are continuously introduced into perinatal and pediatric medicine, and assessment of their relative toxicity is important. The authors compared different methods of assessment of renal damage during development in an attempt to establish their relative sensitivity, age and dose dependence. Newborn, 6- to 8-day-old and adult rats were treated for 7 days with i.m. gentamicin (5, 10 or 20 mg/kg/day) or amikacin (5, 20 or 40 mg/kg/day). Renal damage was assessed by serum and urine creatinine, urine N-acetyl beta-glucosaminidase and beta2-microglobulin, cortical sphingomyelinase in vivo and in vitro and morphol. changes in light and electron microscopy. As expected, there was a dose-dependent damage, with gentamicin being more nephrotoxic than amikacin, and with newborn rats more resistant. The light- and electron- microscopic assessment were more sensitive than all other methods, followed by urinary N-acetyl glucosaminidase and then by beta2-microglobulin. Sphingomyelinase changes occurred only at the highest doses of gentamicin.

162
Sanford WE, Nieboer E. RENAL TOXICITY OF NICKEL IN

HUMANS. Nickel and Human Health: Current Perspectives, E. Nieboer and J. O. Nriagu, Editors; Advances in Environmental Science and Technology, Vol. 25, John Wiley and Sons, Inc., New York, pages 123-134, 43 references, 1992.

The renal toxicity of nickel in humans was discussed. The general characteristics of metal nephrotoxicity were summarized. Metal ion induced renal damage is not a general cytotoxic effect but is specific for individual metals. All metals appear to attack one of two major sites: the proximal convoluted tubule or the glomerulus. A number of studies of nickel workers have found only a few incidences of proteinuria or other biochemical markers of renal dysfunction. Studies in laboratory animals have shown that nickel salts induce proteinuria and hyaline cast formation. Inhalation or injection of nickel-carbonyl has induced transient azotemia that was accompanied by histological evidence of glomerular and tubular damage. The results of a study of kidney function in 26 workers employed in two electrolytic nickel refineries were discussed. The study evaluated kidney function by measuring changes in urinary beta2-microglobulin (b2M), protein, and creatinine excretion and utilizing semiquantitative dipstick tests to detect the presence of ketones, bilirubin, glucose, nitrites, blood, urobilinogen, and protein in urine. Urinary excretion of b2M, total protein, and creatinine was within the normal limits in most workers. Two workers had abnormally low creatinine excretion and three had elevated urine b2M concentrations in individual samples. When the urine b2M concentrations were averaged over three or more samples, urinary b2M excretion of all subjects was within normal limits. Most dipstick tests produced negative results. The few positive test results seen were transient in nature and generally indicated only trace amounts. The authors conclude that the changes in kidney function observed in the nickel refinery workers are minimal when compared to populations exposed to cadmium, a known nephrotoxicant. When compared to the results of studies of other nickel exposed populations, the data indicate that nickel should be regarded as a mild nephrotoxicant.

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Klein J, Koren G, McLeod SM. COMPARISON OF METHODS FOR PREDICTION OF NEPHROTOXICITY DURING DEVELOPMENT. Dev Pharmacol Ther 1992; 19(2-3):80-9.

Drugs with nephrotoxic potential are continuously introduced into perinatal and pediatric medicine, and assessment of their relative toxicity is important. We compared different methods of assessment of renal damage during development in an attempt to establish their relative sensitivity, age and dose dependence. Newborn, 6- to 8-day-old and adult rats were treated for 7 days with intramuscular gentamicin (5, 10 or 20 mg/kg/day) or amikacin (5, 20 or 40 mg/kg/day). Renal damage was assessed by serum and urine creatinine, urine N-acetyl beta-glucosaminidase and beta 2-microglobulin, cortical sphingomyelinase in vivo and in vitro and morphologic changes in light and electron microscopy. As expected, there was a dose-dependent damage, with gentamicin being more nephrotoxic than amikacin, and with newborn rats more resistant. The light- and electron-microscopic assessment were more sensitive than all other methods, followed by urinary N-acetyl glucosaminidase and then by beta 2-microglobulin. Sphingomyelinase changes occurred only at the highest doses of gentamicin.

NEUROTOXICITY

164

Ueda Y, Walsh E, Nakanishi H, Yoshida K. A COLORIMETRIC ASSAY METHOD FOR THE EVALUATION OF NEOROTROPHIC ACTIVITY IN VITRO. Neurosci Lett 1994;165(1-2):203-7.

A colorimetric assay was established to detect neurotrophic activity by measuring the lysosomal enzyme, acid phosphatase (AP) activity of cultured neuronal cells. Neurons from the cerebral cortex of 14- or 15-day mouse embryo were cultured in serum-free medium for 3 days in 96-well culture plates. A linear relation was obtained between the AP activity and the no. of viable neurons counted under a microscope. The AP assay was used to evaluate the neurotrophic activity of basic fibroblast growth factor. This assay is shown to be simple, sensitive and convenient to detect neurotrophic activity.

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Walum E, Nordin M, Beckman M, Odland L. CELLULAR METHODS FOR IDENTIFICATION OF NEUROTOXIC CHEMICALS AND ESTIMATION OF NEUROTOXICOLOGICAL RISK. Toxicol in Vitro 1993;7(4):321-6.

No abstract.

166
Fountain SB, Rowan JD. DEVELOPMENT OF AN IN VITRO HIPPOCAMPAL BRAIN SLICE SCREEN FOR NEUROTOXICITY. Altern Methods Toxicol 1993;9, ISS In Vitro Toxicology: Tenth Anniversary Symposium of CAAT:27-40.

The authors tested a no. of chem. of known in vivo neurotoxic potential. The authors report results of

expts. designed to use the hippocampal slice prepn. to assess the neurotoxic potential of acrylamide and related compounds, lead compounds, and methylmercury. The authors also examined the suitability of methods that might prove effective in obtaining dose-response information using within-slice measurements to speed assessment and to reduce animal use. The results continue to favor the view that an in vitro hippocampal slice screen may prove to be a valid method for neurotoxicity screening.

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Becking GC, Boyes WK, Damstra T, MacPhail RC. ASSESSING THE NEUROTOXIC POTENTIAL OF CHEMICALS: A MULTIDISCIPLINARY APPROACH. Govt Reports Announcements & Index (GRA&I), Issue 23, 1993.

Since 1981, the development of methodology to assess the neurotoxic potential of chemicals has been a high priority within the International Programme on Chemical Safety (IPCS). Following the completion of an in-depth review of the scientific principles and methods for the assessment of neurotoxicity associated with chemical exposures, IPCS started to develop a multidisciplinary and comprehensive approach for neurotoxicity testing of chemicals. In view of the complexity of the nervous system and the variety of effects caused by chemicals, no single test or approach will be appropriate. Initially, IPCS evaluated neurobehavioral tests as well as in vitro procedures as screening tests, and an international collaborative study of neurobehavioral tests appropriate for screening chemicals for neurotoxicity is now in progress. Possible integration of higher level neurobehavioral tests with neurophysiological, biochemical, and pathological procedures in future testing strategies are discussed. (Copyright (c) 1993 Academic Press, Inc.). Journal article. Pub. in Environmental Research, v61 n1 p164-175 Apr 93. Presented at the International

Symposium on Neurobehavioral Methods and Effects in Occupational and Environmental Health (4th), Tokyo, Japan, July 8-11, 1991. Prepared in cooperation with National Inst. of Environmental Health Sciences, Research Triangle Park, NC.

168
Veronesi B, Ehrich M. USING NEUROBLASTOMA CELL LINES TO EXAMINE ORGANOPHOSPHATE NEUROTOXICITY. Govt Reports Announcements & Index (GRA&I), Issue 22, 1993.

The paper describes the initial characterization of neuroblastoma cell lines to address several aspects of organophosphate neurotoxicity. Several commercially available human and mouse cell lines (i.e., SY5Y, IMR-32, SK-N-MC, NB41A3) were evaluated for their target esterase activities (i.e., cholinesterase, neurotoxic esterase, carboxylesterase), and of these cells, a human (SY5Y) and mouse (NB41A3) neuroblastoma cell line clone were used to establish an IC50 cytoxicity profile for a variety of organophosphates insecticides (e.g., parathion, paraoxon, diisopropylphosphorofluoridate and mipafox). The human neuroblastoma cell line clone (SY5Y) was further used to distinguish between neuropathy-causing OPs and cholinesterase inhibitors. These initial data support the use of neuroblastoma cell lines as effective test models for organophosphate neurotoxicity. Journal article. Pub. in In vitro Toxicology: A Jnl. of Molecular and Cellular Toxicology, v6 n1 p57-65 1993. Prepared in cooperation with Virginia-Maryland Regional Coll. of Veterinary Medicine, Blacksburg, VA.

169
Reinhardt CA. NEURODEVELOPMENTAL TOXICITY IN VITRO: PRIMARY CELL CULTURE MODELS FOR SCREENING AND RISK ASSESSMENT. Reprod Toxicol 1993;7(Supp1):165-170. (48REFS)

In-vitro models for assessing neurodevelopmental toxicity were discussed. The general types of models used for identifying teratogens were considered. Robust in-vitro models for assessing neurodevelopmental toxicity were reviewed. These include brain slice preparations, micromass cultures, aggregate cultures under gyratory movement, aggregate cultures derived from chick embryo neural cell cultures, and a system consisting of high cell density monolayers combined with reaggregated cells derived from embryonic chick

brains (ED6 system). The ED6 system was described. The ED6 system was based on monitoring cellular development and differentiation of astrocytes and nerve cells. Changes in glial fibrillary acidic protein and 68 kilodalton neurofilament protein concentration and tyrosine-hydroxylase activity were used as outcome measures. Cytotoxicity was assessed by the neutral-red-dye uptake tests. The results of a validation study of the ED6 system were discussed. The system was used to screen 16 chemicals including four known human teratogens six potential teratogens, and six nonteratogens. The ED6 assay correctly classified 14 compounds. The author concludes that a battery of robust in-vitro systems may be required in order to cover major events leading to neurodevelopmental toxicity.

170
Scott SA, Davies AM. AGE-RELATED EFFECTS OF NERVE GROWTH FACTOR ON THE MORPHOLOGY OF EMBRYONIC SENSORY NEURONS IN VITRO. J Comparat Neurology 1993;

337(2):277-285.

Studies of neonatal and adult mammals have shown that neuronal morphology is regulated in part by the availability of target-derived neurotrophic factor. To test whether the same is true for embryonic neurons, which are dependent on target-derived neurotrophic factors for survival, we grew neural crest-derived sensory neurons from the trigeminal ganglion of avian embryos of different ages in vitro in different concentrations of nerve growth factor (NGF) and measured the number of branch points and total length of the resulting aborizations. Although the size and complexity of arborizations increased with embryonic age up to embryonic day (E)14, neuronal morphology for embryos younger than E14 was unaffected by the concentration of NGF in the culture medium. However, beginning at E14, the stage at which trigeminal neurons start to lose their absolute requirement for NGF for survival, the neurons had significantly more branch points and larger arborizations in higher concentrations of NGF. Thus, it appears that the extent of neurite outgrowth in young embryos is independent of neurotrophic factor concentration; each neuron that receives enough neurotrophic factor to survive elaborates approximately the same size arbor. As trigeminal neurons mature and become less dependent on neurotrophic factor for survival, they acquire the

ability to respond to neurotrophic factor with increased neurite growth and branching, as in neonates and adults.

171
Monnet-Tschudi F, Zurich M-G, Schiller B, Honegger P. AGGREGATE CELL CULTURES OF FETAL RAT TELENCEPHALON A MODEL TO STUDY THE EFFECTS OF CHRONIC EXPOSURE TO NEUROTOXINS. Fourth Meeting of the International Neurotoxicology Association, Helsingor, Denmark, June 6-11, 1993. Neurotoxicology (Little Rock) 1993;14(4):559.

No abstract.

172
Walum E, Nordin M, Beckman M, Odland L. CELLULAR METHODS FOR IDENTIFICATION OF NEUROTOXIC CHEMICALS AND ESTIMATION OF NEUROTOXICOLOGICAL RISK. Toxic In Vitro 1993;7(4):321-326.

This review critically addresses key aspects of neurotoxicity that can be assessed by using in vitro test procedures and batteries. Such test schemes must most probably be hierarchical and multi-optional in order to be able to cope with the large number of possible mechanisms of neurotoxicity. Although the regenerative capacity of the nervous system is low, lesions can be compensated for by a number of cellular dynamic functions (e.g. intracellular calcium sequestration, membrane-bound ion transport systems and increases in the rates of energy metabolism and protein synthesis). Therefore, cellular tests included in primary screens of a multiple system should be based on determinations of cell physiological parameters rather than on measurements of single biochemical reactions. A general neurotoxicity test system for the determination of critical neurotoxic concentrations is suggested to include, as a first step, the assessment of basal cytotoxicity in a human neuroblastoma cell line. In a second step, differential cytotoxicity is assayed in highly developed primary cultures of neuronal and non-neuronal cells. In order to find out whether the compound is likely to produce axonopathy, a test procedure in mouse neuroblastoma cells is carried out. Toxicokinetic information is obtained from hepatocyte/target cell and endothelial cell/astrocyte co-cultures. To disclose alterations in cell physiology, studies of cell respiration, protein

synthesis, membrane permeability and calcium homoeostasis are suggested. When information from these test steps is evaluated together with available data on in vivo toxicity, toxicokinetics and physical/chemical parameters, it may be necessary to proceed to more neuronal specific determinations or mechanistically oriented studies. If a consistent pattern of effects or non-critical and critical concentrations is found, the toxicokinetic distribution over the blood-brain barrier must be considered in relation to actual in vivo blood concentrations if estimates of neurotoxic risk are to be made.

173
Willets JM, Lambert DG, Griffiths HR. SUITABILITY OF B65 AND SH-SY5Y NEUROBLASTOMA CELLS AS MODEL FOR 'IN VITRO' NEUROTOXICITY TESTING. Biochemical Society 647th Meeting on Chromosomal Abnormalites in Cancer Cells: Identificiatio of Molecules Important for Tumour Development, Sheffield, England, UK, July 20-23, 1993. Biochemical Society Transactions 1993;21(4):452S.

No abstract.

174
Hughet F, Piriou A. THE RAT BRAIN SLICE PREPARATION AS AN IN VITRO MODEL OF CHRONIC SYNAPTIC ALTERATIONS. Fourth Meeting of the International Neurotoxicology Association, Helsingor, Denmark, June 6-11, 1993. Neurotoxicology (Little Rock) 1993; 14(4):558.

No abstract.

175
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.

Morphologic lesions have received only limited attention as in vitro endpoints of toxicity. In the present work, "tissue" and cell morphology of control and toxicant-treated primary dissociated cerebrocortical cell cultures from fetal mice were examined 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 containing 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. Chemicals induced neuronotoxic, gliotoxic, and cytotoxic (i.e., nonspecific) patterns of morphologic damage in growing (< 6 day old) or mature (6-15 day old) cultures in both a concentration-dependent and cell type-specific manner. In addition, 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 histopathologic endpoints, including methods for neural-specific markers, represent potentially valuable criteria for in vitro assessments of neurotoxicity.

176
Dodt HU, Hager G, Zieglgansberger W. DIRECT OBSERVATION OF NEUROTOXICITY IN BRAIN SLICES WITH INFRARED VIDEOMICROSCOPY. J Neurosci Methods 1993; 50(2):165-71.

We employed the novel technique of infrared videomicroscopy to study the morphological changes induced by the neurotoxicity of high concentrations of L-glutamateand by anoxia. The infrared videomicroscopy system described uses an inverted microscope and employs a combination of infrared illumination, differential interference contrast (DIC) and contrast enhancement by video. With this system, we were able to observe swelling of neurons 50 microns deep in rat neocortical slices after bath application of glutamatergic agonists or during anoxia. By recording in time lapse mode it was possible to visualize the dynamics of cell swelling and to demonstrate neuroprotection by glutamatergic antagonists. The method may be of use in screening of potential neuroprotective drugs for stroke therapy.

177
Dodt HU, Hager G, Zieglgansberger W. DIRECT OBSERVATION OF NEUROTOXICITY IN BRAIN SLICES WITH INFRARED VIDEOMICROSCOPY. J Neurosci Methods 1993;50(2):165-71.

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videomicroscopy to study the morphological changes induced by the neurotoxicity of high concentrations of L-glutamate and by anoxia. The infrared videomicroscopy system described uses an inverted microscope and employs a combination of infrared illumination, differential interference contrast (DIC) and contrast enhancement by video. With this system, we were able to observe swelling of neurons 50 microns deep in rat neocortical slices after bath application of glutamatergic agonists or during anoxia. By recording in time lapse mode it was possible to visualize the dynamics of cell swelling and to demonstrate neuroprotection by glutamatergic antagonists. The method may be of use in screening of potential neuroprotective drugs for stroke therapy.

178
Willets JM, Lambert DG, Griffiths HR. SUITABILITY OF B65 AND SH-SY5Y NEUROBLASTOMA CELLS AS MODEL FOR 'IN VITRO' NEUROTOXICITY TESTING. Biochemical Society 647th Meeting on Chromosomal Abnormalities in Cancer Cells: Identification of Molecules Important for Tumour Development, Sheffield, England, UK, July 20-23, 1993. Biochemical Society Transactions 1993;21(4):452S.

No abstract.

179
Durham HD, Dahrouge S, Cashman NR. EVALUATION OF THE SPINAL CORD NEURON X NEUROBLASTOMA HYBRID CELL LINE NSC-34 AS A MODEL FOR NEUROTOXICITY TESTING. Neurotoxicology (Little Rock) 1993;14(4):387-395.

NSC-34 is a hybrid cell line produced by fusion of motor neuron enriched, embryonic mouse spinal cord cells with mouse neuroblastoma. Cultures contain two populations of cells: small, undifferentiated cells that have the capacity to undergo cell division and larger, multi-nucleate cells that express many properties of motorneurons. The utility of NSC-34 cells as a model for investigation of neurotoxicity was evaluated following exposure of cultures to a selection of chemicals known to be neurotoxic to motor neurons. NSC-34 responded to agents that affect voltage-gated ion channels, cytoskeletal organization and axonal transport. The sensitivity of action potential production to various ion channel blockers was similar to that in primary motor neurons in culture. 2,5-hexanedione induced focal aggregation of

neurofilaments in perikarya and processes of NSC-34. Sodium pyridinethione induced swelling and retraction of processes. In contrast, NSC-34 was not a good model in which to investigate agents that affect synaptic transmission. No electrophysiological evidence of synaptic connections between NSC-34 cells was obtained. Exposure to 1 mM glutamate had no effect on cell morphology or action potential production. Difficulties in using this line to investigate chemical neurotoxicity were poor substrate adhesion, requirement for routine subculture and change in expression of the neuronal phenotype with repeated subculture.

180
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. Toxicology In Vitro 1993; 7(5):569-580.

Many cell culture models are available for the in vitro assessment of neurotoxicity. The use of three culture types has been investigated: neuroblastoma cell lines, primary cultures of rat and chick midbrain, and organotypic whole brain reaggregate cultures. A tiered system has been proposed involving hierarchical testing through three layers of different neural complexities. This scheme is currently undergoing validation under the auspices of FRAME/EC using 40 test chemicals. To determine the performance and suitability of these culture models studies on selected neurotoxins have been performed: ethylcholine mustard aziridinium, vincristine, aluminium, 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 cytotoxicological 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 chemicals, 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 versus adult nervous systems are considered.

181
Abdulla EM, Campbell IC. L-BMAA AND KAINATE-INDUCED MODULATION OF NEUROFILAMENT CONCENTRATIONS AS A MEASURE OF NEURITE OUTGROWTH: IMPLICATIONS FOR AN IN VITRO TEST OF NEUROTOXICITY. Toxicology In Vitro 1993; 7(4):341-344.

This work shows that the neurotoxic excitatory amino acid analogues beta-N-methyl- L-amino-alanine (L-BMAA) and kainate, modulate neurite outgrowth. This was assessed indirectly by measuring the levels of two different neurofilament proteins (68 kDa and 160 kDa) in a mouse neuroblastoma cell line (NB41A3). The results of this study show that at low doses (10-9-10-7 M) both L-BMAA and kainate decrease the concentration of the two neurofilament proteins but that at high doses (10-6-10-5 M) they cause an apparent accumulation; the effect is more marked with L-BMAA. The sensitivity of the neurofilaments to low doses (10-8 M) of the latter suggests that this test may be useful as a general in vitro test of neurotoxicity. In addition, these in vitro observations may shed light on the formation of the 'neurofibrillary tangles' commonly found in the brains of patients who have had Guam disease and/or Alzheimer's disease.

182
Manger RL, Leja LS, Lee SY, Hungerford JM, Wekell MM. TTRAZOLIUM-BASED CELL BIOASSAY FOR NEUROTOXINS ACTIVE ON VOLTAGE-SENSITIVE SODIUM CHANNELS: SEMIAUTOMATED ASSAY FOR SAXITOXINS, BREVETOXINS AND CIGUATOXINS. Anal Biochem 1993;214(1):190-194.

In the present study we have developed an assay for the detection of sodium channel-specific marine toxins based upon mitochondrial dehydrogenase activity in the presence of veratridine and ouabain. This cell bioassay allows detection of either sodium channel enhancers, such as the brevetoxins and the ciguatoxins, or sodium channel blocking agents, such as the saxitoxins. The assay responds in a dose dependent manner and differentiates the toxic activity as either sodium channel blocking or enhancing. In addition, the assay is highly sensitive, with present detection limits of 2 ng/ml for either saxitoxins or brevetoxins (PbTx-1 and

PbTx-3). Assay response to a ciguatoxic extract and to brevetoxins is rapid, allowing dose dependent detection within 4 to 6 h. The method is simple, utilizes readily available reagents, uses substantially less sample than required for mouse bioassay, and is well within the scope of even modest tissue culture facilities. This cell-based protocol has the potential to serve as an alternate and complementary method to the standard mouse bioassay.

OCULAR TOXICITY

183
Matsumoto SS, Stern ME, Oda RM, Ghosn CR, Cheng JW, Gelber TA, Wang Y, Zoutis AM, Anger CB. EFFECT OF OFLOXACIN ON CORNEAL EPITHELIAL WOUND HEALING EVALUATED BY IN VITRO AND IN VIVO METHODS. Drug Invest 1993; 6(2):96-103.

Ofloxacin, at concns. and times of exposure that were based on in vivo pharmacokinetic data, had little effect on the in vitro proliferation of rabbit corneal epithelial cell cultures. For example, at a concn. of 3 mg/mL for 5 min or 50 mug/mL for 48 h there was little or no effect on in vitro cell proliferation. Functional in vitro cell proliferation assays, such as cell outgrowth from corneal biopsies and wound closure, were less sensitive to ofloxacin than were assays of metabolic redn. or protein synthesis. In an animal model of wound healing, corneal epithelial abrasion wounds in New Zealand albino rabbits were treated with ofloxacin 3 mg/mL 4 times daily at 4-h intervals. The epithelial healing rate was the same for ofloxacin-treated eyes and control eyes treated with phosphate buffered saline. These results support the safety of ofloxacin on the healing cornea.

184
McCulley JP, Stephens TJ. DRAIZE EYE TESTING ALTERNATIVES. A PERSPECTIVE. Altern Methods Toxicol 1993; 9(In Vitro Toxicology: Tenth Anniversary Symposium of CAAT):107-19. (17 REFS)

No abstract.

185
Kristen U, Kappler R, Pape WJ W, Hoppe U. IN VITRO TOXICITY ASSESSMENT OF TENSIDES - THE POLLEN TUBE GROWTH TEST, THE RED BLOOD CELL TEST AND THE DRAIZE EYE

IRRITATION ASSAY IN COMPARISON. BioEngineering (Graefelfing, Fed Repub Ger) 1993; 9(5):39-45.

The recently developed in vitro pollen tube growth test (PTG test) for the detection of cytotoxicity of bioactive chem. was compared with 2 established assays, the Draize test and the red blood cell test (RBC test). Both latter methods are suited to est. irritation potentials of tensides and detergents. Comparison of the corresponding dose response data of all 3 assays, using 22 tensides as test substances, revealed highly significant rank correlation between PTG test and the Draize test, but relatively low correlation between RBC test and PTG test.

186
Decker D, Stemp M, Harper R. EVALUATION OF THE EYTEX SYSTEM FOR USE AS A PREDICTOR OF OCULAR IRRITANCY: II. CONDITIONERS AND STYLING AIDS. J Toxicol Cutan Ocul Toxicol 1993;12(4):371-380.

The Eytex in vitro assay was used to evaluate 33 opaque and clear conditioners and 48 styling aids including hairsprays, mousses, styling gels, and lotions. The assay, which operates on the principle of protein precipitation, is quantitative and relatively inexpensive. Two different protocols were used: the rapid membrane assay (RMA) protocol for the conditioners, mousses, styling gels, and lotions; and the upright membrane assay (UMA) for the hairsprays. All samples were tested in two or more separate experiments and the scores averaged. One hundred percent of the conditioners, 91% of the hairsprays, and 87% of the other styling aids produced qualified Eytex scores. Irritation classes established previously for shampoos were used to determine correlation to Draize eye irritation categories. When the Draize eye irritation class was compared to the Eytex irritation class for a given qualified sample, the correlation was 1.0 for the opaque conditioners, 0.14 for the clear conditioners, 0.87 for hairsprays, and 1.0 for the other styling aids. This study indicates that the Eytex in vitro assay for ocular irritancy can be highly predictive of Draize eye scores for opaque conditioners and styling aid products. Such assays could be useful as a screening tool in new product development.

187
Catroux P, Rougier A, Dossou KG, Cottin M. THE SILICON

MICROPHYSIOMETER FOR TESTING OCULAR TOXICITY IN VITRO.
Toxicol In Vitro 1993;7(4):465-469.

The silicon microphysiometer has been used for in vitro evaluation of the ocular irritancy potential of water soluble ingredients and formulations. This light-addressable potentiometric sensor detects changes in cell physiology by monitoring the rate at which cultured cells excrete their acidic products of metabolism. We have mainly determined the metabolic effect of 53 products (21 surfactants and 32 surfactant-based formulations). The related maximal average Draize score (MAS) were available from historical data and varied from 1.7 to 54. All of the Draize categories were represented. Murine fibroblastic cells (L929 clone) were exposed to increasing concentrations of the product for approximately 400 sec per dose. The MRD50 (dose of product that decreased the metabolic rate of the cells by 50%) was determined by interpolation from a plot of metabolic rate versus test material concentration. Decreases in metabolic rate, as assessed by the MRD50, occurred over a wide range of concentrations (40 mug/ml-200 mg/ml). The linear (Pearson) and rank (Spearman) correlation between in vivo (MAS) and in vitro (log MRD50) data were 0.91 and 0.89, respectively. This study indicates that the silicon microphysiometer method exhibits a high correlation with the Draize test for water-soluble raw materials and formulations and thus can be used as an in vitro screen for ocular irritation.

188
Jones PA. MICROTOX AND IN VITRO TESTING FOR OCULAR IRRITATION POTENTIAL. Richardson, M. (ED.). Ecotoxicology Monitoring; International Symposium, London, England, UK, June 17, 1992. XXV+384P. VCH Verglassgesellschaft MBH: Weinheim, Germany; VCH Publishers, Inc.: New York, New York, USA. ISBN 3-527-28560-1; ISBN 1-56081-736-4.; 0 (0). 1993. 261-268.

No abstract.

189
Kahn CR, Young E, Lee IH, Rhim JS. HUMAN CORNEAL EPITHELIAL PRIMARY CULTURES AND CELL LINES WITH EXTENDED LIFE SPAN: IN VITRO MODEL FOR OCULAR STUDIES.
Invest Ophthalmol Vis Sci 1993;34(12):3429-41.

PURPOSE. To develop an in vitro model of human corneal epithelium that can be propagated in serum-free medium that is tissue specific, species specific, and continuously available. METHODS. Primary explant cultures from human cadaver donor corneas were generated and subsequently infected with Adeno 12-SV40 (Ad12-SV40) hybrid virus or transfected with plasmid RSV-T. RESULTS. Several lines of human corneal epithelial cells with extended life span were developed and characterized. Propagation of both primary cultures and lines with extended life span, upon collagen membranes at an air-liquid interface, promoted multilayering, more closely approximating the morphology observed in situ. CONCLUSIONS. In vitro models, using primary cultures of corneal epithelium and lines of corneal epithelial cells with extended life span, retain a variety of phenotypic characteristics and may be used as an adjunct to ocular toxicology studies and as a tool to investigate corneal epithelial cell biology.

190
Sina JF, Gauthoron PD. OCULAR TOXICITY ASSESSMENT IN VITRO. Gad, S. C. (ED.). In Vitro Toxicology. IX+290P. Raven Press: New York, New York, USA. ISBN 0-88167-974-7. 1994; 0(0):21-46.

No abstract.

191
Catroux P, Rougier A, Dossou KG, Cottin M. THE SILICON MICROPHYSIOMETER FOR TESTING OCULAR TOXICITY IN VITRO. Toxicol in Vitro 1993;7(4):465-9.

The silicon microphysiometer has been used for in vitro evaluation of the ocular irritancy potential of water sol. ingredients and formulations. This light-addressable potentiometric sensor detects changes in cell physiol. by monitoring the rate at which cultured cells excrete their acidic products of metab. The authors have mainly detd. the metabolic effect of 53 products (21 surfactants and 32 surfactant-based formulations). The related maximal av. Draize score (MAS) were available from historical data and varied from 1.7 to 54. All of the Draize categories were represented. Murine fibroblastic cells (L929 clone) were exposed to increasing concns. of the product for approx. 400 s per dose. The MRD50 (dose of product that decreased the metabolic rate of the cells by 50%) was

detd. by interpolation from a plot of metabolic rate vs. test material concn. Decreases in metabolic rate, as assessed by the MRD50, occurred over a wide range of concns. (40 mug/mL-200 mg/mL). The linear (Pearson) and rank (Spearman) correlation between in vivo (MAS) and in vitro (log MRD50) data were 0.91 and 0.89, resp. This study indicates that the silicon microphysiometer method exhibits a high correlation with the Draize test for water-sol. raw materials and formulations and thus can be used as an in vitro screen for ocular irritation.

ORGAN CULTURE

192
Stahlmann R, Klug S, Foerster M, Neubert D.
SIGNIFICANCE OF EMBRYO CULTURE METHODS FOR STUDYING THE PRENATAL TOXICITY OF VIRUSTATIC AGENTS.
Reprod Toxicol 1993;7(Suppl 1):129-143. (38 REFS)

Culture methods have become important tools for elucidating the prenatal toxicity of drugs and other xenobiotics. In this paper the authors review in vitro as well as in vivo approaches to demonstrate the teratogenic potential of aciclovir and other related virustatic agents. In addn., some new data on this topic are given. The teratogenic potential of the virustatic agent aciclovir was not recognized in routinely performed segment-II-studies, but the first indication came from expts. with the culture of rat embryos. Subsequently, the findings were confirmed in modified in vivo tests and it became clear that out of a group of six related drugs aciclovir exhibited the highest potential for prenatal toxicity. The effects of aciclovir on limb development were not pronounced; this has been shown with in vitro and in vivo expts. as well. In vivo expts. first indicated that the prenatal development of the thymus is disturbed by aciclovir. This effect was further studied with the culture of fetal thymuses and again the effect of aciclovir could be compared with related drugs. In summary, the authors' work with virustatics during the last years has shown that in vivo and in vitro approaches are by no means competitive and that a combination of both approaches can provide a solid basis for a toxicol. evaluation.

193
Berman E, Laskey JW. ALTERED STEROIDOGENESIS IN WHOLE-OVARY AND ADRENAL CULTURE IN CYCLING RATS.

Reprod Toxicol 1993;7(4):349-58.

Cultures of minced, whole-ovary (whole-ovary culture) were used to det. if 3 selected chemicals altered steroidogenic profiles. First, phenolsulfonthalein (PST), when used in culture medium, was tested for its influence on in vitro steroidogenesis. Next, aminoglutethimide (AGTP; 0 or 150 mg/kg once) and di(2-ethylhexyl)phthalate (DEHP; 0 or 1500 mg/kg/day for 10 days) were administered in vivo to young adult cycling rats, and the ovaries and adrenals were removed and cultured for 1 h. Ovarian steroidogenic profiles of progesterone (P), testosterone (T), and estradiol (E) release into the medium were measured using RIA techniques. PST in medium significantly decreased ovarian P prodn. and altered T and E prodn. so that the T/E ratio was significantly altered. Therefore, PST was excluded in the later studies. DEHP altered steroid profiles so that proestrus appeared to be delayed. AGTP

decreased P and E prodn. significantly, and T prodn. was increased slightly in proestrus ovaries. These AGTP alterations in T and E resulted in a highly significant increase in the T/E ratio. Adrenals from the DEHP and AGTP expts. were also cultured for 1 h, and P was assayed in the medium. AGTP, but not DEHP, significantly increased the prodn. of P in adrenals. Whole-ovary culture is recommended as an in vitro test for chems. suspected of interfering with steroidogenesis in vivo. This test model should be placed strategically between in vivo studies of reproductive toxicity and complex in vitro mechanistic studies.

194
Jansen HT, Cooke PS, Porcelli J, Liu T-C, Hansen LG. ESTROGENIC AND ANTIESTROGENIC ACTIONS OF PCBS IN THE FEMALE RAT: IN VITRO AND IN VIVO STUDIES. Reprod Toxicol 1993;7(3):237-248. (41 REFS)

The estrogenic and antiestrogenic actions of polychlorinated biphenyls (PCBs) as individual congeners and complex mixtures were investigated in the female rat using both in-vitro and in-vivo methods. The PCBs used were Arochlor-1242 (A1242), 3,4,3',4'-tetrachlorobiphenyl (PCB77), 2,5,2',5'-tetrachlorobiphenyl, and 2,4,6-trichloro-4'-hydroxy-biphenyl (14962288). Anterior pituitaries of young female Sprague-Dawley-rats were cultured, and test compounds

or 17-beta-estradiol (E2) were added and incubated with or without gonadotropin releasing hormone (GnRH). Luteinizing-hormone and follicle stimulating hormone levels in both media and cytosols were measured using radioimmunoassay. In in-vivo studies the test compounds were injected with or without E2, as single doses or as repeated doses, and uterine weights and tritiated thymidine (3H-Thy) uptake were measured. Results showed that except for PCB77, all PCBs and E2 produced significant increases in uterine weights as well as increases in 3H-Thy labeling. PCB77 caused an attenuation of the uterine weight increase caused by A1242 or E2 treatment. The in-vitro results showed that varying concentration of A1242 caused enhanced gonadotropin responses to GnRH similar to that of E2. The authors conclude that PCBs can produce both estrogenic and antiestrogenic effects.

195
Berman E, Laskey JW. ALTERED STEROIDOGENESIS IN WHOLE OVARY AND ADRENAL CULTURE IN CYCLING RATS. Repord Toxicol 1993;7(4):349-358.

Cultures of minced, whole-ovary (whole-ovary culture) were used to determine if three selected chemicals altered steroidogenic profiles. First, phenolsulfonthalein (PST), when used in culture medium, was tested for its influence on in vitro steroidogenesis. Next, aminoglutethimide (AGTP; 0 or 150 mg/kg once) and di(2-ethythexyl)phthalate (DEHP; 0 or 1500 mg/kg/day for 10 days) were administered in vivo to young adult cycling rats, and the ovaries and adrenals were removed and cultured for 1 h. Ovarian steroidogenic profiles of progesterone (P), testosterone (T), and estradiol (E) release into the medium were measured using radioimmunoassay techniques. PST in medium significantly decreased ovarian P production and altered T and E production so that the T/E ratio was significantly altered. Therefore, PST was excluded in the later studies. DEHP altered steroid profiles so that proestrus appeared to be delayed. AGTP decreased P and E production significantly, and T production was increased slightly in proestrus ovaries. These AGTP alterations in T and E resulted in a highly significant increase in the T/E ratio. Adrenals from the DEHP and AGTP experiments were also cultured for 1 h, and P was assayed in the medium. AGTP, but not DEHP, significantly increased the production of P in adrenals. Whole-ovary culture is recommended as an in vitro test for chemicals suspected of interfering with

steroidogenesis in vivo. This test model should be placed strategically between in vivo studies of reproductive toxicity and complex in vitro mechanistic studies.

PULMONARY TOXICITY
196
Niven RW, Lott FD, Ip AY, Somaratne kD, Kearney M.
DEVELOPMENT AND USE OF AN IN VITRO SYSTEM TO EVALUATE INHALER DEVICES. Int J Pharm 1994;101(1-2):81-7.

An in vitro system was developed to better emulate particle deposition in the respiratory tract. Inhalers were connected to a glass throat (BP 1988 Appendix XVII C) or to a silicone throat that exactly duplicated the surface geometry of the oral and pharyngeal cavity. This throat was created from a direct impression of the mouth and CAT scans of a patient's head and neck and could be sepd. into three parts. This allowed deposition patterns to be obsd. Adapters were fabricated so that the outlet of either throat could be connected to a collection filter unit or any one of several sizing instruments. A mass flow meter enabled airflow to be monitored. Airflow was produced from a vacuum pump or by human inhalation. A Rotahaler was tested using capsules contg. 20 mg of spray dried mannitol:sorbitol:carboxyfluorescein (CF) 10:1:0.01 of 3.4 mum MMAD. The vacuum pump was set at 30, 60 or 120 l/min air flow for 4 s. The surface of the glass or silicone throat was left 'dry' or was coated with a polyethylene glycol mixt. to better represent the 'wet' surface of the oral cavity. Powder in the device, filter unit and throat(s) was quantified by assay of the CF using spectrofluorimetry. The recovery of the weighed dose was 98.6 .+-. 8.9% (n = 83). The dose emerging from the inhaler was dependent on the flow rate and was 1.0 .+-. 0.2 mg (30 1/min, n = 25), 5.1 .+-. 0.5 mg (60 1/min, n = 30) and 6.2 .+-. 0.6 mg (120 1/min, n = 28). However, the percentage of this dispensed dose recovered from the filter unit (lung) was independent of the flow rate and only varied with the type and condition of the throat used. The mass deposition in the throats was ranked: glass-dry < silicone-dry < glass-wet < silicone-wet. The results indicate that the use of a wet artificial throat, modeled on human anatomy, will provide a more conservative est. of lung deposition compared to a glass throat when used with a dry powder inhaler.

197
Bordenave L, Bareille R, Rouais F, Lefebvre F, Amedee J, Baquey C, Janvier G, Pellet F. HUMAN TRACHEAL EPITHELIAL CELLS IN CULTURE: A SUITABLE MODEL FOR TESTING THE CYTOCOMPATIBILITY OF MATERIALS FOR ENDOTRACHEAL USE. J Mater Sci: Mater Med 1993; 4(3):327-36.

It is well known that cuffs of endotracheal tubes can induce ischemic injuries on tracheal epithelium, as a result of mech. hyperpressure caused by the cuff on the airway tissue. Whether or not material components are leached out and may provoke a direct toxic effect on the respiratory epithelium is much less clear. To study the cytocompatibility of such materials, the authors have developed an in vitro cell system using human tracheal epithelial cells, arising from trachea superficial biopsies. In culture, cells have been characterized by morphol. and immunocytochem. criteria. Ultrastructural observations suggest that the authors' culture conditions are permissive for the expression of both squamous and secretory phenotypes. The authors have assessed the cytocompatibility of a cuff towards epithelial cells, first, by an indirect test, and second by a direct test. By the indirect test, using material exts., the authors did not find any toxic effect towards human airway epithelial cells of the cuff components. By a direct test, the authors found a slight cell lysis after a 24 h incubation. The authors' study shows that this human tracheal epithelial cell system is a useful and relevant model which could be used in a quality control procedure for testing the cytocompatibility of materials for endotracheal use.

198
Bruch J, Rehn B, Song W, Gono E, Malkusch W. TOXICOLOGICAL INVESTIGATIONS ON SILICON CARBIDE. 2. IN VITRO CELL TESTS AND LONG TERM INJECTION TESTS. British Journal of Industrial Medicine 1993; 50(9):807-813. (35 REFS)

The pneumotoxic potential of silicon-carbide was studied in-vivo and in-vitro. Female Wistar-rats were injected intratracheally with 50 milligrams (mg) silicon-carbide dust. Other rats were injected intratracheally with 2mg quartz dust for comparison. They were killed 3 or 8 months later and the lungs and mediastinal lymph nodes were removed. The lymph nodes were weighed. The lungs were examined for histopathological changes. Alveolar macrophages

isolated from guinea-pig lungs were incubated with 0 to 100 micrograms (microg) silicon-carbide, quartz, and tempered clay. The effects on macrophage mediated release of hydrogen-peroxide and tumor necrosis factor alpha (TNFa) activity against L929 tumor cells were determined. Quartz at 60microg completed inhibited release of hydrogen-peroxide from guinea-pig macrophages. The 20microg dose caused a 40% inhibition of hydrogen-peroxide release. Neither silicon-carbide nor tempered clay inhibited macrophage mediated hydrogen-peroxide release. Quartz caused a dose dependent suppression of TNFa activity against L929 cells. Silicon-carbide and tempered-clay did not affect TNFa activity. Following intratracheal injection, silicon-carbide caused only slight, nonsignificant increases in mediastinal lymph node weight. Lungs from silicon-carbide exposed rats showed only changes typical of inert dust deposition. No areas of collagen development, indicative of fibrotic activity, were observed. Quartz induced significant increases in mediastinal lymph node weight. The authors conclude that silicon-carbide does not produce lung tissue damage in laboratory animals. The previous findings of radiographic abnormalities in workers occupationally exposed to silicon-carbide could reflect simultaneous exposures to other substances.

199
Donaldson K, Miller BG, Sara E, Slight J, Brown RC. ASBESTOS FIBRE LENGTH-DEPENDENT DETACHMENT INJURY TO ALVEOLAR EPITHELIAL CELLS IN VITRO: ROLE OF A FIBRONECTIN-BINDING RECEPTOR. International J Experiment Pathol 1993;74(3):243-250. (34 REFS)

The role of fiber length in amosite (12172735) induced epithelial cell injury was examined. Human-A549 cells, a type 2 alveolar epithelial cell line, were incubated with up to 100 micrograms (microg) amosite samples that had less than 10% of the fibers longer than 10 microns (short fibers) or had approximately 70% of the fibers 10 microns or longer (long fibers) for 4 hours. All fibers had similar diameters. In some experiments the fibers had been pretreated with poly-L-lysine (PLL), desferal, serum, glutathione, catalase, superoxide-dismutase (SOD), or rat lung lining fluid or precoated with fibronectin. Other A549 cells were incubated with 1microg per milliliter (ml) phorbol- myristate-acetate (PMA). The degree of cell injury was assessed by determining the extent of cell detachment from the substratum. The extent of cellular lysis was determined

using the chromium-51 release assay. Long amosite fibers caused significantly more cell detachment than short fibers. Induction of cell detachment by the long fibers increased with dose up to 25microg, then tended to plateau. None of the fibers induced cell lysis. PLL, desferal, serum, glutathione, catalase, SOD, and rat lung lining fluid did not alter the effect of the long fibers. Precoating with fibronectin inhibited the ability of the long fibers to induce cell detachment. PMA at concentrations up to 0.1microg/ml induced cell detachment in a dose dependent manner. Higher concentrations caused a slight decrease in the extent of cell detachment. The authors conclude that long amosite fibers can detach human alveolar epithelial cells from their substratum. This detachment injury differs from the classical asbestos induced injury in that it does not involve lysis and is not ameliorated by desferal, an iron chelator, or antioxidants. The fibronectin binding receptor or the second messenger system of the cells could be involved in inducing this type of injury.

200
Lannan S, Donaldson K, Brown D, Macnee W. EFFECT OF CIGARETTE SMOKE AND ITS CONDENSATES ON ALVEOLAR EPITHELIAL CELL INJURY IN VITRO. American J Physiology 1994;266(1 PART 1):L92-L100.

The oxidant-antioxidant balance in the airspaces of the lungs may be critical in protecting the lungs from the effects of cigarette smoke. We studied the effect of cigarette smoke and its condensates on the detachment, attachment, and proliferation of the A549 human alveolar epithelial cell line, in an in vitro model of cell injury and regeneration and the protective effects of antioxidants. Whole and vapor phase cigarette smoke decreased 51Cr-labeled A549 cell attachment, increased cell detachment, and decreased cell proliferation, as assessed by (3H)thymidine uptake. Freshly isolated rat type II alveolar epithelial cells showed an enhanced susceptibility to smoke-induced cell lysis when compared with the A549 cell line. Reduced glutathione (GSH) (400 muM) protected against the effects of cigarette smoke exposure on cell attachment, proliferation, and detachment. Depletion of intracellular GSH with buthionine sulfoxamine enhanced the epithelial cell detachment injury produced by smoke condensates. We conclude that cigarette smoke and its condensates cause an oxidant-induced injury to A549 human type II alveolar epithelial cells. Both intra-

and extracellular GSH have important roles in protecting epithelial cells from the injurious effects of cigarette smoke.

201
Nemery B, Hoet PH. USE OF ISOLATED LUNG CELL IN PULMONARY TOXICOLOGY. Toxicol In Vitro 1993; 7(4):359-364.

Although there are many possible approaches to the study of chemical-induced lung injury, it has become clear in recent years that it is important, particularly for mechanistic studies, to clarify the responses of specific target cells in the lungs. The alveolar macrophage has been extensively studied because of its important role in the response of the lung to inhaled dusts and in the development of inflammatory lung disorders, and because it is a cell type that can be easily obtained from animals and humans by the technique of bronchoalveolar lavage. The type II cell and the Clara cell have important physiological functions and they are considered to be the main sites for the biotransformation of xenobiotics in the lung. In recent years methods have been developed to isolate, purify and culture these cells from laboratory animals. The greatest promise of these in vitro techniques is in the possibility of studying human cells, and thus coming closer to scientifically sound extrapolations of experimental data to the human situation.

202
Hurbankova M, Kaiglova A. ALVEOLAR MACROPHAGES AS INDICATORS IN THE TESTING OF THE EFFECTS OF SOME FIBROUS AND NONFIBROUS DUSTS IN VITRO. Pracovni Lekarstvi 1993;45(5):208-212.

No abstract.

203
Ciabattoni G, Montuschi P, Curro D, Preziosi P. IN VITRO TESTING FOR LUNG TOXICITY. Toxic In Vitro 1993; 7(5):581-585.

The authors characterized the pattern of arachidonic acid metabolites released from sensitized isolated guinea pig lungs undergoing anaphylactic reactions after ovalbumin challenge. TXB2 (the stable hydrolysis

product of TXA2) is the most abundant product: it was released at an average of 13.7 : 7.0 ng/min (: SD, n = 33) during the anaphylactic reaction (i.e. a five- to six-fold increase in comparison with the basal release). Its level correlates with both the bronchoconstrictor response and LTC4 and LTB4 release. In non-sensitized lungs the release of basal TXB2 increased about five-fold during a 10-min exposure to a formaldehyde aerosol at the dose of 10 ppm; this increase was concomitant with an 87.5 : 10.0% (: SD) reduction of the tracing recording the pressure-volume variations in the lung. No change in the release of lipoxygenase products was observed after exposure to formaldehyde aerosol. Pretreatment with N-acetylcysteine (10-3-10-4 M) reduced both formaldehyde-induced bronchoconstriction and the increase of TXB2 release. An acid-water aerosol, resembling the composition of acid rain, strongly increased TXB2 release in the pH range 4.5 to 2.5, without affecting the lipoxygenase pathway of arachidonic acid metabolism. These data suggest that exposure to toxicants that irritate the respiratory tract selectively enhances TXB2 release, while an antigen challenge stimulates both the cyclooxygenase and lipoxygenase pathways of lung arachidonate metabolism. Thus, the different pattern of arachidonic acid cascade activation may predict a direct toxic effect or an allergic reaction when the xenobiotic is injected into the pulmonary artery or administered by way of inhalation as an aerosol. More recently vasoactive intestinal polypeptide (VIP) has been proposed as a modulator of lung inflammation and airway constriction. In sensitized lungs the antigen challenge induced a five-fold increase in VIP release, coinciding with the increase of arachidonate metabolites. Pharmacological evidence shows that TXA2 enhances VIP release, while VIP suppresses TXA2 formation. This suggests a possible role of TXA2/VIP interaction in regulating bronchial smooth muscle reactivity in condition of enhanced arachidonate metabolism. However, the importance of this interaction in evaluating the toxic response is still to be established.

204
Hadnagy W, Seemayer NH, Happel A, Kiell A. HUMAN ONOCYTE-DERIVED MACROPHAGE CULTURES: AN ALTERNATIVE TEST SYSTEM FOR THE DETECTION OF PULMONARY TOXICITY INDUCED BY INHALED PARTICULATE POLLUTANTS. Toxicol In Vitro 1993;7(4):365-371.

Alveolar macrophages are major target cells for toxicity from inhaled particulates. To investigate pulmonary toxicity induced by airborne particulates a phagocytosis assay using human monocyte-derived macrophage cultures was used. Monocytes were isolated from peripheral blood and cultured for 10-14 days. During this period the monocytes differentiated to macrophages. After treatment with various concentrations of different samples of airborne particulate matter, phagocytosis was induced by the addition of Polychromatic Fluoresbrite Microspheres at a cell/particle ratio of 1:10. Phagocytosis was assessed from the determination of phagocytic activity (% cells showing phagocytosis) and of phagocytic capacity (number of phagocytized particles/cell). A concentration-dependent reduction of phagocytic activity and capacity was observed, while cell viability was not greatly affected as compared with the control. These results were in good agreement with those obtained from in vivo inhalation experiments with rodents as well as with those using rat alveolar macrophages obtained after bronchoalveolar lavage and treated in vitro with extracts of particulate matter. Therefore, it is suggested that the use of human monocyte-derived macrophage cultures represents a promising in vitro test system for the evaluation of pulmonary toxicity induced by particulate pollutants.

205
Li L, Lau BH S. A SIMPLIFIED IN VITRO MODEL OF OXIDANT INJURY USING VASCULAR ENDOTHELIAL CELLS. In Vitro Cell Dev Biol 1993: Anim;29A,(7):531-6.

In the present study, a convenient in vitro model of oxidant injury induced by hydrogen peroxide (H2O2) was developed using bovine pulmonary artery endothelial cells (PAEC). Viability of PAEC grown in 96-well culture plates was detd. with methylthiazol tetrazolium (MTT) colorimetric assay. Cell membrane integrity was measured by lactate dehydrogenase (LDH) release from PAEC grown in 24-well plates. Malondialdehyde (MDA, a product of lipid peroxidn.) in PAEC grown in 6-well plates was detected by a thiobarbituric acid fluorometric assay. Incubation of H2O2 with PAEC caused a dose-dependent decrease of cell viability, an increase of LDH release, and an elevation of MDA prodn. MTT assay was convenient, quant., non-radioactive, and suitable for testing a large no. of samples. The fluorometric assay for measuring MDA prodn. in endothelial cells used 6-well plates instead of 80-cm2

flasks employed by previous investigators. The use of multiwell culture plates in these assays made it possible for more samples to be tested in any single expt. The three assays are reproducible with low intraplate and interplate coeffs. of variation. This in vitro model is suitable for screening antioxidants and for studying pharmacodynamics at the cellular level.

REGULATORY TOXICOLOGY
206
Koeter H B WM. TEST GUIDELINE DEVELOPMENT AND ANIMAL WELFARE REGULATORY ACCEPTANCE ON IN-VITRO STUDIES. International Workshop on In Vitro Methods in Reproductive Toxicology, Ottawa, Ontario, Canada, May 19-20, 1992. Reprod Toxicol 1993;7(SUPPL 1):117-123.

No abstract.

REPRODUCTIVE TOXICITY
207
Lamb JC, Chapin RE. TESTICULAR AND GERM CELL TOXICITY: IN VITRO APPROACHES. Reprod Toxicol 1993;7(Supp 1): 17-22.

In-vitro approaches for investigating testicular and germ cell toxicity were discussed. The general principles and methods used in in-vitro and in-vivo testicular toxicity studies and their relevancy for human risk assessment were reviewed. In-vitro systems have the disadvantage that they can miss one or more steps in the initial toxifying or detoxifying stages of the spermatogenetic process. In-vitro test systems can be used alone or in combination with in-vivo studies to test hypotheses about testicular toxicity. Advantages of in-vitro test systems for investigating testicular toxicity were discussed. In-vitro systems are very useful for investigating possible active toxic metabolites and examining their metabolic activity in target cells. In-vitro systems can be used to screen a class of compounds for germ cell toxicity if the target cell or process is known. Such screening can be useful for setting priorities for risk assessment. The disadvantages of in-vitro test systems included loss of structural integrity of the entire organ or system, loss of paracrine interactions, and inducing artefactual changes in the metabolic activity of target or adjacent cells. Changes in some spermatogenetic processes are difficult to evaluate in-vitro. Specific toxic effects are also difficult to evaluate in some

cases because cells in many in-vitro testicular systems have very limited life spans. The authors conclude that in-vitro test systems can be very helpful in assessing testicular and germ cell toxicity; however, they do have limitations. In-vitro systems are best suited for screening classes of compounds, testing metabolites, identifying cellular targets, and investigating molecular mechanisms of action.

208
Mattison DR. SITES OF FEMALE REPRODUCTIVE VULNERABILITY: IMPLICATIONS FOR TESTING AND RISK ASSESSMENT. Reprod Toxicol 1993;7(Supp 1): 53-62. (112 REFS)

Testing chemicals for female reproductive system toxicity was discussed. Techniques used for general reproductive toxicity evaluations include computer assisted evaluations of structure activity relationships (SARs), in-vitro assays, and laboratory animal experiments. SAR evaluations can be used to rapidly and inexpensively screen a large number of chemicals. Their relevancy to reproductive toxicity depends on the parameters used to establish and validate the SAR predictions. In-vitro assays can be used to characterize the site and mechanisms of reproductive toxicity. They represent useful adjuncts to in-vivo assays and SAR evaluations. Laboratory animal assays may be the most expensive and time consuming, but they represent the most complete technique for characterizing the reproductive toxicity of the test chemical. The general principles for assessing female reproductive system toxicity risks were considered. Target sites for chemical injury in the female reproductive system were reviewed. These include the hypothalamus, anterior pituitary, ovary, granulosa cells, thecal cells, and oocytes. Relationships between various measures of female fecundity and fertility were discussed. The potential impact of a chemical toxicant on fertility can be examined by determining the cumulative percentage of females that have become pregnant, cycle specific fertility rates, or the mean number of cycles to conception. Quantitative estimates of reproductive risk can be obtained by determining the magnitude of the change in any of these parameters induced by exposure to a toxicant.

209

Tariq M. A REVIEW OF REPRODUCTIVE TOXICITY OF ALUMINIUM. Richardson, M. (ED.). Reproductive Toxicology. XIX+286P. VCH Verglassesellschaft MBH: Weinheim, Germany; VCH Publishers, Inc.: New York, New York, USA. ISBN 3-527-28561-X; ISBN 1-56081-737-2.; 0 (0). 1993. 245-256.

No abstract.

210
Li S. REPRODUCTIVE TOXICOLOGY CHINA. Richardson, M. (ED.). Reprod Toxic. XIX+286P. VCH Verlagsgesellschaft MBH: Weinheim, Germany; VCH Publishers, Inc.: New York, New York, USA. ISBN 3-527-28561-X; ISBN 1-56081-737-2. 1993;0(0):63-71.

No abstract.

211
Waysbort A, Giroux M, Mansat V, Teixeira M, Dumas JC, Puel J. EXPERIMENTAL STUDY OF TRANSPLACENTAL PASSAGE OF ALPHA INTERFERON BY TWO ASSAY TECHNIQUES. Antimicrob Agents Chemother 1993 Jun;37(6):1232-7.

Two methods of assaying alpha interferon (IFN-alpha) were compared during an experiment aimed at determining whether IFN-alpha crosses the human placenta. Human placentas, collected after delivery following a normal pregnancy to term, were catheterized on both sides: fetal and maternal. The IFN-alpha was introduced in known amounts in the maternal circulation and was assayed in the efferent fetal fluid. The following two detection methods were used: radioimmunoassay by competition with [125I]IFN-alpha and assay with a biological system in which IFN-alpha protected Madin-Darby bovine kidney cells from destruction by vesicular stomatitis virus. The results obtained by the two methods were in perfect agreement for the efferent fetal fluid samples. They showed the absence of placental transfer of IFN-alpha. The biological method was found to be more sensitive than radioimmunoassay for low IFN-alpha titers (< 10 IU/ml) but was less reproducible, probably owing to the use of twofold dilutions. The specificities of the two methods were similar and their practicalities were equivalent; the biological method, however, was less costly. The study illustrates the complementarity of the two methods, which were based on different principles. The agreement obtained between the two methods provides a clear

confirmation of the experimental results.

212
Ueda O, Suzuki H, Matsuoka A, Sugiyama O, Adachi J. AN APPLICATION OF IN VITRO FERTILIZATION--EMBRYO CULTURE--EMBRYO TRANSFER SYSTEM ON THE DRUG SAFETY EVALUATION: FERTILITY TEST OF MALE MICE ADMINISTERED WITH THE ANTICANCER DRUG. Jikken Dobutsu 1993;42(4):565-70.

We examined an application of in vitro fertilization--embryo culture--embryo transfer system for reproductive and developmental study on the drug safety evaluation in mice. The male mice at 10 weeks of age were administered intravenously with a single dose of 75 mg/kg of the anticancer platinum complex (DWA 2114R) which inhibits DNA synthesis. Four to six weeks after administration, the males were mated with the superovulated females. Fertilization rates were significantly lower than the controls at each weeks after the administration. Furthermore, delayed formation of pronucleus was observed as compared with the control. Four weeks after administration, the preimplantation development to blastocyst stage of those embryos in vitro and the survival rates on the day 17 of gestation after embryo transfer suggested that a DNA synthesis in germ cells during maturation was inhibited and/or prevented by DWA 2114R. The results of in vitro fertilization reflected its sperm concentration rather than the administration of DWA 2114R. Thus, an analysis of the delayed formation of pronucleus observed fertilization in vivo could not done in detail. To use for the drug safety evaluation, there exist plenty of room for improvement in this system. These results have showed that the embryo culture and the embryo transfer are useful techniques as the reproductive and developmental study on the drug safety evaluation. These techniques bring additional informations on the pre- and post-implantation development in vivo.

213
Palmer AK. IDENTIFYING ENVIRONMENTAL FACTORS HARMFUL TO REPRODUCTION. Environ Health Perspect 1993;101 (Sup.2):19-25.

Reproduction is essential for the continuation of the species and for life itself. In biological terms, living and reproducing are essentially one and the

same. There is, therefore, no sharp division between identifying factors harmful to reproduction and identifying factors harmful to life or vice versa. Detection of harmful factors requires balanced use of a variety of methodologies from databases on structure-activity relationships through in vitro and in vivo test systems of varying complexity to surveys of wildlife and human populations. Human surveys provide the only assured means of discriminating between real and imagined harmful factors, but they are time consuming and provide information after the harm has been done. Test systems with whole animals provide the best prospects for identifying harmful factors quickly, but currently available methods used for testing agrochemicals and drugs need a thorough overhaul before they can provide a role model. Whether there is a need for new methodology is doubtful. More certain is the need to use existing methodology more wisely. We need a better understanding of the environment--whatever it is--and a more thoughtful approach to investigation of multifactorial situations.

214
Schmid BP, Honegger P, Kucera P. EMBRYONIC AND FETAL DEVELOPMENT FUNDAMENTAL RESEARCH. International Workshop on In Vitro Methods in Reproductive Toxicology, Ottawa, Ontario, Canada, May 19-20, 1992. Reprod Toxicol 1993;7(SUPPL. 1):155-164.

No abstract.

215
Mummery CL, Slager HG, Van Inzen W, Freund E, Van Den Eijnden-Van Raaij A JM. REGULATION OF GROWTH AND DIFFERENTIATION IN EARLY DEVELOPMENT OF MICE AND MODELS. INTERNATIONAL WORKSHOP ON IN VITRO METHODS IN REPRODUCTIVE TOXICOLOGY, OTTAWA, ONTARIO, CANADA, MAY 19-20, 1992. Reprod Toxicol 1993;7(SUPPL 1):145-154.

No abstract.

216
Christian MS. IS THERE ANY PLACE FOR NONMAMMALIAN IN-VITRO TESTS? International Workshop on In Vitro Methods in Reproductive Toxicology, Ottawa, Ontario, Canada, May 19-20, 1992. Reprod Toxicol 1993;7(SUPPL 1):99-102.

No abstract.

217
Palmer AK. INTRODUCTION TO PRESCREENING METHODS. International Workshop on In Vitro Methods in Reproductive Toxicology, Ottawa, Ontario, Canada, May 19-20, 1992. Reprod Toxicol 1993;7(SUPPL 1): 95-98.

No abstract.

218
Jarrell JF, Sevcik ML, Villeneuve DC, Janson PO. TOXICITY TESTING USING THE ISOLATED IN-VITRO PERFUSED OVARY. International Workshop on In Vitro Methods in Reproductive Toxicology, Ottawa, Ontario, Canada, May 19-20, 1992. Reprod Toxicol 1993;7(SUPPL 1):63-68.

No abstract.

219
Comhaire FH. METHODS TO EVALUATE REPRODUCTIVE HEALTH OF THE HUMAN MALE. International Workshop on In Vitro Mehtods in Reproductive Toxicology, Ottawa, Ontario, Canada, May 19-20, 1992. Reprod Toxicol 1993;7(SUPPL 1):39-46.

No abstract.

220
Steinberger A, Klinefelter G. SENSITIVITY OF SERTOLI AND LEYDIG CELLS TO XENOBIOTICS IN IN-VITRO MODELS. International Workshop on In Vitro Mehtods in Reproductive Toxicology, Ottawa, Ontario, Canada, May 19-20, 1992. Reprod Toxicol 1993;7(SUPPL 1):23-37.

No abstract.

RESPIRATORY TOXICITY
221
Montano LM, Jones GL, O'Byrne PM, Daniel EE. EFFECT OF OZONE EXPOSURE IN VIVO ON RESPONSE OF BRONCHIAL RINGS IN VITRO: ROLE OF INTRACELLULAR CALCIUM. J Appl Physiol 1993;75(3):1315-1322.

In this study we investigated the role of intracellular Ca2+ in ozone- (O3) induced airway hyperresponsiveness.

Acetylcholine- induced airway responses were measured before and after inhalation of O2 (3 ppm, 30 min) or dry air. In vitro experiments were performed with intact ring segments of third- to fifth-order bronchi. Bronchial responses to carbachol (CCh) were evaluated in Krebs solution (2.5 mM Ca2+) and in Ca2+-free (0.1 mM ethylene glycol-bis(beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA)) Krebs solution with or without indomethacin (IDM, 10-5 M) and were expressed as percentage of the maximal KCl response (60 mM). Inhalation of O3, but not dry air, caused airway hyperresponsiveness to acetylcholine in vivo. Responses to 50% effective concentrations of CCh were similar in bronchial preparations from O3 and control animals (with or without IDM) in normal Krebs solution. In Ca2+-free solution, CCh induced a sustained (20-min) bronchial contraction. These contractions relaxed immediately when nifedipine or a high EGTA concentration was added to the organ bath. The sustained contraction was abolished when the tissues had been incubated with cyclopiazonic acid (10-5 M), a novel inhibitor of the sarcoplasmic reticulum Ca2+ pump. After O3 exposure, responses of the bronchial smooth muscle (in Ca2+-free medium without IDM) were increased ($P < 0.05$) compared with controls during the first and second CCh stimulations. This O2-induced increase in response to CCh in Ca2+-free solution was abolished when the tissues were incubated with IDM. Thus, changes in the control and apparent recycling of intracellular Ca2+ in bronchial smooth muscle and its release by CCh may be involved in O3-induced airway hyperresponsiveness, and prostanoids affect this system.

STRUCTURE/ACTIVITY TOXICITY

222
Ashby J. TWO MILLION RODENT CARCINOGENS? THE ROLE OF SAR AND QSAR IN THEIR DETECTION. Mutat Res 1994; 305(1):3-12. (17 REFS)

The accurate prediction of chemical carcinogenicity can only be achieved by a balanced consideration of the following factors: the chem. and metab. of the test agent, the interaction between toxicity and genetic toxicity, the possibility of non-genotoxic events that trigger subsequent non-targeted mutagenesis, the difference between activities obsd. in vitro and in vivo, and the possible inadequacy and/or partiality of all datasets and observations. Extrapolation of activities within a series of congeners is usually

possible, but predictions across different chemical classes/mechanisms of carcinogenicity are difficult. Artificial intelligence systems can be used to predict one or more of the above parameters given adequate learning sets, but the hope for a single, coherent and self-contained method of predicting all instances of carcinogenicity is unreal. The future of carcinogen/mutagen prediction lies with data-rich artificial intelligence systems based on known mechanistic principles used selectively within the context of chemical and biological human insight. The major current obstacle to progress is the assumption that mutagenicity and carcinogenicity are unitary phenomena that can be learned and predicted by artificial intelligence systems operating in isolation.

223
Ashby J. INTERNATIONAL COMMISSION FOR PROTECTION AGAINST ENVIRONMENTAL MUTAGENS AND CARCINOGENS. TWO MILLION RODENT CARCINOGENS? THE ROLE OF SAR AND QSAR IN THEIR DETECTION. Mutat Res 1994;305(1):3-12. (REFS: 17)

The accurate prediction of chemical carcinogenicity can only be achieved by a balanced consideration of the following factors: the chemistry and metabolism of the test agent, the interaction between toxicity and genetic toxicity, the possibility of non-genotoxic events that trigger subsequent non-targeted mutagenesis, the difference between activities observed in vitro and in vivo, and the possible inadequacy and/or partiality of all datasets and observations. Extrapolation of activities within a series of congeners is usually possible, but predictions across different chemical classes/mechanisms of carcinogenicity are difficult. Artificial intelligence systems can be used to predict one or more of the above parameters given adequate learning sets, but the hope for a single, coherent and self-contained method of predicting all instances of carcinogenicity is unreal. The future of carcinogen/mutagen prediction lies with data-rich artificial intelligence systems based on

known mechanistic principles used selectively within the context of chemical and biological human insight. The major current obstacle to progress is the assumption that mutagenicity and carcinogenicity are unitary phenomena that can be learned and predicted by artificial intelligence systems operating in isolation.

224
Kavlock RJ. STRUCTURE-ACTIVITY APPROACHES IN THE SCREENING OF ENVIRONMENTAL AGENTS FOR DEVELOPMENTAL TOXICITY. International Workshop on In Vitor Methods in Reproductive Toxicology, Ottawa, Ontario, Canada, May 19-20, 1992. Reprod Toxicol;7(SUPPL 1):113-116.

No abstract.

225
Enslein K. THE FUTURE OF TOXICITY PREDICTION WITH QSAR. In Vitro Toxic 1993;6(3):163-169.

Quantitative structure-activity relationships (QSAR) for chemicals were first demonstrated almost a century ago. In recent years QSAR has been applied to modelling of toxicity endpoints. Current models are mainly limited by the lack of sufficient high-quality data, the fact that metabolism and pharmacokinetics are generally not taken into account, and the need to interactively validate the resulting predictions. Remedies to these limitations are outlined, including greater exploitation of 3-D descriptors, the use of fuzzy logic, and the application of metabolism and pharmacokinetics principles. The potential of QSAR in toxicology will include reduction of number of animals used for toxicity revaluation, optimization of therapeutic ratios for pharamceuticals and other bioactive chemicals, improvement in design of bioassays, and assistance in the elucidation of mechanisms of action. There is also the potential for better extrapolation of predictions to humans, and integration of predictions from multiple modalities into a single, more meaningful overall evaluation of a chemical's toxicity potential.

TERATOGENICITY
226
Flint OP. IN VITRO TESTS FOR TERATOGENS: DESIRABLE ENDPOINTS, TEST BATTERIES AND CURRENT STATUS OF THE MICROMASS TERATOGEN TEST. Reprod Toxicol 1993;7(Supp 1):103-111. (47 REFS)

The micromass test for assessing teratogenicity was discussed. The micromass teratogen test is an in-vitro bioassay that utilizes cultured chick, mouse, or rat embryo midbrain and limb cells to examine the effects of the test agent on cellular differentiation. Problems

typically encountered in applying in-vitro test systems to risk assessments were considered. Procedures for preparing cells for the micromass assay utilizing rat embryos were described. Analyzing and interpreting data obtained in the micromass test was discussed. The ability of the micromass test to detect embryotoxicity and its sensitivity to teratogens were considered. The capability of the micromass test to predict in-vivo teratogenicity was discussed. The micromass test has been shown to be capable of detecting 60 to 90% of known in-vivo teratogens and 89 to 100% of the nonteratogens. Very little interlaboratory variability in obtaining these results has been observed. Most of the variability can be explained by the test configuration selected, the compounds tested, and the length of exposure to the test agent. The author concludes that the micromass teratogen test is a robust in-vitro bioassay for detecting potential teratogens.

227
Vismara C, Bernardini G, Bonfanti P, Colombo A, Camatani M. THE USE OF IN VITRO FERTILIZATION IN THE FROG EMBRYO TERATOGENESIS ASSAY IN XENOPUS FETAX AND ITS APPLICATIONS TO ECTOTOXICOLOGY. Science of the Totol Environment 1993;0 (SUPPL. PART 1):787-790.

No abstract.

228
Newman LM, Johnson EM, Haghdoost NR. A TOXIC AND TERATOGENIC POTENTIAL RANKING OF SODIUM ARSENATE, SODIUM ARSENITE, AND CACODYLIC ACID BY THE APPLICATION OF THE IN VITRO HYDRA ASSAY. Teratology 1993; 47(5):430-1.

Concerns exist that human exposure to arsenicals may cause some early embryonic losses. Studies in pregnant mammals established: higher toxicity and teratogenicity of unmethylated arsenics over methylated arsenics, higher potency of sodium arsenite (Asi) over sodium arsenate (Asa). The current study was undertaken to rank Asa, Asi, and the methylated form, cacodylic acid (Cac), both in terms of developmental toxicity hazard potential and potency, as well as to identify the endpoint times (targets of toxicity) in adult and developing hydra. Results: Asa, Asi, and Cac exposure resulted in the adult (A)/developmental (D) ratios of 11, 10, and 6.5, respectively. The minimal

concentration of the chemicals resulting in the toxic endpoints (death of adult and developing hydra) occurred at 18, 26, and 90 hrs for Asi, Asa, and Cac, respectively. End-point concentrations consisted of: Asa (A = 90 mg/L, D = 8 mg/L), Asi (A = 20 mg/L, D = 2 mg/L), Cac (A = 2,000 mg/L, D = 300 mg/L). Conclusion: The decrease of A/D ratio (indicating increased sensitivity of the hydra "embryo") observed for Cac in comparison to those of Asa and Asi and the higher potency of Asi over Asa and Cac describe the developmental toxicity status of each chemical in hydra. End-point times suggest that each chemical may have a different target during development. The delayed end-points of Cac may be related to the effects of the diminished toxic potential due to the methylated configuration, or a change in the action site. The same consideration could be applied to responses seen in Asa and Asi. Hydra not only ranked these agents, but also indicated differences in developmental mechanisms which can be studied further in this pilot system.

TISSUE CULTURE

229
Lake BG, Beamand JA, Japenga AC, Renwick A, Davies S, Price RJ. INDUCTION OF CYTOCHROME P-450-DEPENDENT ENZYME ACTIVITIES IN CULTURED RAT LIVER SLICES. Food Chem Toxicol 1993;31(5):377-386.

Xenobiotic induction of cytochrome-P-450 dependent mixed function oxidase activities was studied in cultured rat liver slices. Phenobarbitone (PB), beta-naphthoflavone (BNF), and Aroclor-1254 (ARO) were chosen for study. Precision cut liver slices were prepared from male Sprague-Dawley-rats and cultured in conditioned RPMI-1640 medium for up to 72 hours with daily dosing with test medium. Cytochrome-P-450 content in the slices declined to 36% of the levels present in freshly cut rat liver slices after 48 hours in culture. BNF or ARO additions prevented to some degree the loss of cytochrome-P-450 from the slices. PB, BNF, and ARO in the culture medium induced 7-ethoxycoumarin-O-deethylase, 7-benzoxyresorufin- O-debenzylase and 7-ethoxyresorufin-O-deethylase activities. In the 72 hour cultures the induction of mixed function oxidase enzymes was generally greater than in the 48 hour cultures. ARO produced greater stimulation of the enzyme activities at the concentrations studied than did either PB or BNF. The authors conclude that rat liver slices may be maintained in culture for up to 72 hours, and that they respond in a manner similar to rat

primary hepatocyte cultures to some inducers of xenobiotic metabolism. They suggest that the use of precision cut liver slices may be an alternative in-vitro system to hepatocyte cultures for screening compounds and for assessing species differences in response.

230
Meldgaard T, Tonder N, Finsen BR, Lehrman E, Zimmer J. ORGANOTYPIC SLICE CULTURES AN IN VITRO MODEL FOR RECEPTOR MEDIATED NEUROTOXICITY. Fourht Meeting of the International Neurotoxicology Association, Helsingor, Denmark, June 6-11, 1993. Neurotoxicology (Little Rock) 1993;14(4):558.

No abstract.

231
Fisher R, Brendel K, Hanzlik RP. CORRELATION OF METABOLISM, COVALENT BINDING AND TOXICITY FOR A SERIES OF BROMOBENZENE DERIVATIVES USING RAT LIVER SLICES IN VITRO. Chem-Biol Interact 1993;88(2-3):191-208.

For many acute-acting chemicals, toxic responses observed in vivo correlate strongly with metabolic activation and macromolecular covalent binding (CVB) observed in vitro and often in vivo; bromobenzene (BB) is a classic example of this behavior. Substituent groups modulate the toxicity of bromobenzene in vivo and in liver slices cultured in vitro in parallel fashion (Fisher, R., Hanzlik, R.P., Gandolfi, J.A., and Brendel, K. (1992), In Vitro Toxicology, 4, 173-186). In the present study we used the liver slice system to examine the relationship between toxicity, metabolism and covalent binding amongst a series of (3H/14C) dual labelled BB derivatives including (in order of increasing hepatotoxicity) o-bromoanisole (BA), o-bromotoluene (BT), o-bromobenzonitrile (BBN), BB and o-dibromobenzene (DBB). Among these congeners apparent relative rates of metabolism varied only 4-fold, but the most extensively metabolized compounds were the least toxic. CVB varied 7-fold across the series, and those compounds which bound the most frequently were the most toxic. For each compound the relative binding index (RBI = pmol bound/nmol metabolized) and the average retention of tritium relative to carbon-14 in the CVB fraction were constant throughout the 24 h incubations, suggesting that the metabolic profile of each compound remained constant with time. The RBI

values, however, did not reflect relative toxicity as well as total CVB values. The T/C ratios of the CVB residues varied from 0.36 (for BA) to 0.81 (for BBN), indicating that ortho- substitution on BB exerts important qualitative as well as quantitative effects on overall metabolism and reactive metabolite formation. The finding that relative toxicity among a series of bromobenzene congeners is paralleled by their relative covalent binding measured in the same system in which toxicity is assessed adds support to the hypothesis that covalent binding contributes to the observed toxicity, rather than merely being a correlated epiphenomenon.

TOXICOLOGY (GENERAL)

232

Parnham MJ,Wetzig H. TOXICITY SCREENING OF LIPOSOMES. Chem Phys Lipids 1993;64(1-3):263-74.

Phospholipids are the major components of most liposomes. Extensive testing of these naturally occurring compounds has revealed them to be remarkably safe for pharmaceutical use. Addition of other constituents to liposomes in order to alter stability or kinetics can result in an increase in toxic potential, particularly on parenteral administration of liposomes. This paper describes some simple in vitro cellular tests for direct toxicity of liposomes, particularly following i.v. or topical administration, including tests for hemolysis, thrombosis and cytotoxicity. In additionn, an in vivo test for the effects on phagocytosis and for pyrogenicity are described, together with a brief outline of the requirements for the further toxicity testing of liposomal drugs at a later stage of development.

233

Calleja MC, Persoone G, Geladi P. HUMAN ACUTE TOXICITY PREDICTION OF THE FIRST 50 MEIC CHEMICALS BY A BATTERY OF ECOTOXICOLOGICAL TESTS AND PHYSICOCHEMICAL PROPERTIES. 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 determine the acute toxicity of the 50 priority chemicals of the Multicentre Evaluation of In Vitro Cytotoxicity (MEIC)

programme. These tests and five physiocochemical properties (n-octanol-water partition coefficient, molecular weight, melting point, boiling point and density) are evaluated either singly or in combination to predict human acute toxicity. Acute toxicity in human is expressed both as oral lethal doses (HLD) and as lethal concentrations (HLC) derived from clinical 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 ecotoxicological and rodent tests) is generally superior to that of individual physicochemical properties for HLD. For HLC prediction, however, no consistent trend could be discerned that indicated whether bioassays are better estimators than physicochemical 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 ecotoxicological tests and physicochemical properties, the battery based on the molecular weight and the cladoceran crustacean predicts HLC substantially better than any other combination.

234
Guzzie PJ. LETHALITY TESTING. Gad, S. C. (ED.). In Vitro Toxicology. IX+290P. Raven Press: New York, New York, USA. ISBN 0-88167-974-7.; 0 (0). 1994. 57-86.

No abstract.

TUMOR
235
Fuchsberger N, Kubes M, Kontsek P, Borecky L, Hornak M, Godal A, Silvanova, Svec J. IN VITRO ANTIPROLIFERATIVE EFFECT OF INTERFERON ALPHA IN SOLID TUMORS: A POTENTIAL PREDICTIVE TEST. Neoplasma 1993;40(5):293-6.

An in vitro test for the antiproliferative effect of

human leukocyte interferon (IFN-alpha) was performed in primary cultures of tumor cells obtained from 32 patients with either malignant melanoma (13), renal carcinoma (4) or bladder carcinoma (15). Our results demonstrated activity of IFN in all three groups of solid tumors. However, appreciable differences in sensitivity to antiproliferative effect of IFN between individual tumors of the same type were found. The potential of this antiproliferative test for prediction of treatment response in IFN-therapy is discussed.

VALIDATION TESTS

236

Ellard S, Parry JM. A COMPARATIVE STUDY OF THE USE OF PRIMARY CHINESE HAMSTER LIVER CULTURES AND GENETICALLY ENGINEERED IMMORTAL V79 CHINESE HAMSTER CELL LINES EXPRESSING RAT LIVER CYP1A1, 1A2 AND 2B1 CDNAS IN MICRONUCLEUS ASSAYS. Toxicology 1993;82(1-3):131-49.

Liver microsome prepns.(S9 mix) have been extensively used for in vitro genotoxicity studies to provide the capacity for the activation of indirect genotoxins. However, the use of S9 prepns. with mammalian cell cultures has raised considerable toxicity problems which limit their use to exposure times which are only a small fraction of the cell cycle. In addition, false negative results may be obtained if reactive metabolites are unable to penetrate the cell membrane or have short half-lives. The generation and detection of a promutagen within a single cell would therefore be advantageous. To this end, the authors have studied the bioactivation of a panel of promutagens (benzo[a]pyrene, cyclophosphamide, 2-aminoanthracene and sterigmatocystin) in low passage Chinese hamster cell lines genetically engineered to express rat liver cytochrome P 450 cDNAs. These include strains XEM2 (expresses CYP1A1), SD1 (CYP2B1) and strains XEMd-MZ and XEMd-NH which express CYP1A2. The end point selected for study was the induction of micronuclei. The protocol incorporated a cytochalasin B-induced cytokinesis block and the enumeration of micronuclei in the resulting binucleate cells which have undergone one

nuclear division following the induction of chromosome damage. Micronuclei contg. whole chromosomes and chromosome fragments were distinguished by the use of CREST antibody specific for kinetochore protein as a measure for the presence of centromeres. Micronuclei were induced by the test agents in low passage liver fibroblasts and in immortal V79 cultures only in the

presence of Aroclor-induced S9 prepns. The data obtained for micronucleus assays of the genetically engineered V79 cell lines demonstrate the utility of each strain for the optimal detection and quantification of the activity of the individual test compds. Kinetochore antibody demonstrated differences in the kinetics of induction of micronuclei contg. chromosome fragments and whole chromosomes with chems. such as benzo[a]pyrene. As part of this cytogenetic study, the authors also conducted karyotypic analyses and spindle fidelity assays of the V79 cell lines to investigate the presence of chromosomal instabilities which may arise as a consequence of the genetic engineering procedure. Such studies represent an important quality control step in the validation of suitability of each cell line prior to their use in genotoxicity studies.

237
Rasmussen ES. THE ROLE OF IN VITRO EXPERIMENTS IN ANIMAL WELFARE. Human & Experimental Toxicology 1993;12(6):522-527.

The prospects of replacing animal experiments with other types of toxicological studies are considered, and the use of human data and in vitro experiments are discussed. Ongoing validation studies of in vitro methods for evaluation of acute toxicity, local irritation, target organ toxicity, tumour promotion and teratogenicity are presented.

238
Green S. REGULATORY AGENCY CONSIDERATIONS AND REQUIREMENTS FOR VALIDATION OF TOXICITY TEST ALTERNATIVES. Toxicol Lett 1993;68(1-2):119-23.

When developing an alternative toxicity test, one must first determine whether the alternative assay is to be used as a screen or as a replacement for the traditional toxicity test. An assay used as a screen will require less stringent acceptance criteria, for it is designed to answer fewer and less complex questions (e.g., the assessment of only potential teratogenicity). An assay used as a replacement will be used to establish hazard or lack thereof (safety). In other words, a replacement assay must clearly establish whether or not a chemical is a teratogen. One should

also have knowledge of and experience with the in vivo assay to be replaced. This knowledge should be of not only the procedural aspects of the test but also the regulatory information it provides (i.e., how the results are used for hazard determination). Thorough consideration of the regulatory information is critical for a test intended to be used as a replacement. Validation should include intralaboratory and interlaboratory reproducibility of results from a standard protocol, an assessment of the qualitative and quantitative aspects of the test responses, and the use of a sufficient number of chemicals representative of the defined category of interest.

239
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. Toxic In Vitro 1993;7(4):505-510.

According to OECD guideline 405 revised in 1987 Draize eye tests need not be performed for severely irritating and corrosive chemicals 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 programme, 136 chemicals from the German chemical 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. Chemicals were, therefore, selected as representatives of chemical structural groups as well as of

physicochemical and toxicological properties. In addition, some of the chemicals 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 interlaboratory assessment trial, it was impossible to correlate cytotoxicity data to the EEC classification for in vivo eye irritation. However, seven of 10 severely irritating chemicals (EEC labelling 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 chemicals (EEC labelling 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-labelled' chemicals in the HET-CAM test is encouraging, for safety assessment of non-irritant chemicals, 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.

XYZ/MISCELLANEOUS

240

Fischbach M, Sabbioni E, Bromley P. INDUCTION OF THE HUMAN GROWTH HORMONE GENE PLACED UNDER HUMAN HSP70 PROMOTER CONTROL IN MOUSE CELLS: A QUANTITATIVE INDICATOR OF METAL TOXICITY. Cell Biol Toxicol 1993;9(2):177-88.

An in vitro test method for general metal toxicity screening was designed, based on the cellular response to stress. The expression of a transfected human growth hormone gene sequence driven by the human heat-shock protein 70 promoter in NIH/3T3 cells was used as marker of noxious contact with metal compounds. Out of a series of 31 metals, 17 were competent for inducing this stress response system. According to the effective concentration and to the intensity of the response, three different clusters of positive compounds emerged and were ranked as strong, intermediate strength and weak inducers. These results correlated well with data from other in vivo and in vitro metal toxicity studies, including LD50 in mice. Apparently the positive/negative compounds also fitted well with data from genotoxicity and carcinogenesis studies on metal salts.

241

Sampson HA. IN VITRO DIAGNOSIS AND MEDIATOR ASSAYS FOR FOOD ALLERGIES. Allergy Proc 1993;14(4):259-61. (REFS: 10)

No abstract.

242
Zheng H, Shah PK, Audus KL. PRIMARY CULTURE OF RAT GASTRIC EPITHELIAL CELLS AS AN IN VITRO MODEL TO EVALUATE ANTIULCER AGENTS. Pharm Res 1994;11(1):77-82.

Primary rat gastric cell cultures were investigated as an in vitro model for evaluating antiulcer agents. Following exposure to concentrations of up to 5 mg/mL of an antiulcer agent sucralfate, an aluminum hydroxide complex of sucrose octasulfate, cultured cells were treated with either pH 3.5 medium or 3.5 mM indomethacin. Cytoprotection was evaluated by colony forming efficiency, neutral red uptake, and 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) hydrolysis. By each measure, and depending on damaging agent, 2 and 5 mg/mL sucralfate provided partial (50% of untreated control) to near-complete (90% of untreated control) cytoprotection, respectively. Aluminum hydroxide also provided partial (55% of untreated control) to near-complete (more than 90% of untreated control) cytoprotection at 2 and 5 mg/mL, respectively, for the pH 3.5 medium-induced damage. Over a concentration range of 0.05 to 5 mg/mL, the potassium salt of sucrose octasulfate, KSOS, stimulated cell growth up to 40-60% over untreated controls but had little or no cytoprotective action in the presence of either 3.5 mM indomethacin or pH 3.5 medium. Overall results suggested that sucralfate may have at least two roles in influencing gastric epithelial cell function, cytoprotection and stimulation of cell growth in vitro. These observations serve as a basis for further study of in vitro models in evaluating the cytoprotective activity of antiulcer agents and their respective mechanisms of action.

243
Oshiro Y, Kachur JF, Balwierz PS, Won-Kim S. AN IN VITRO SYSTEM TO SCREEN FOR DIARRHEAGENIC CHEMICALS. Cell Biol Toxicol 1993;9 (1):85-94.

We examined an in vitro system to screen for diarrheagenic chemicals using an established intenstinal cell line (T84 human colonic carcinoma). The cells were grown on Millicell-PCF (polycarbonate membrane) wells. The cells were seeded at approximately 5ated for 9-11 days in a 5% CO2 incubator saturated with water at 37~ C. The culture medium was a 1:1 mixture of Ham's F12 and Dulbecco's

MEM with 5% fetal bovine serum and 25 mug/ml gentamicin sulfate. The well containing cells was removed from the incubator and mounted in a modified Ussing chamber for measurement of short-circuit current (ISC). Chemical-induced increases in ISC are usually indicative of electrogenic epithelial Cl- secretion, which is associated with diarrhaegenic effects in animals and humans. T84 cells grown on Millicell-PCF membrane responded with an increase in ISC after basolateral addition of the cholinergic (muscarinic) agonist carbachol, prostaglandin E2, 16,16-dimethylprostaglandin E2, and forskolin, while non-diarrheagenic prostaglandin D2 did not affect ISC. Based on our results, this in vitro system has the potential to be adapted as a rapid screen for detecting diarrheagenic chemicals.

244
Fritzenschaf H, Kohlpoth M, Rusche B, Schiffmann D. TESTING OF KNOWN CARCINOGENS AND NONCARCINOGENS IN THE SYRIAN HAMSTER EMBRYO (SHE) MICRONUCLEUS TEST IN VITRO: CORRELATIONS WITH IN VIVO MICRONUCLEUS FORMATION AND CELL TRANSFORMATION. Mutat Res 1993; 319(1):47-53.

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

245
Voegeli R, Meier J, Doppler S. DEFENCE STRATEGIES AGAINST REACTIVE OXYGEN SPECIES: IN VITRO MODELS. Int

J Cosmet Sci 1993; 15(4):153-161.

A simple in vitro test system was established to determine the degradation of hyaluronic acid induced by reactive oxygen species (ROS). ROS were selectively generated by exogenic and endogenic factors and the effects of directly and indirectly acting antioxidants with different modes of action were systematically examined. The proposed experimental strategy, simulating specific stress conditions, can be used for an easy and fast screening of putative antioxidant activity of water soluble compounds. Under the chosen test conditions, the antioxidant enzymes catalase and Cu/Zn-superoxide dismutase revealed the most effective protection against the ROS-induced depolymerization of hyaluronic acid, whereas individual tested compounds acted as prooxidants under certain circumstances.

246
Brusick DJ. IN VITRO TOXICOLOGY GREAT EXPECTATIONS. In Vitro Toxicology 1993;6(3):137-140.

No abstract.

247
Balls M. HOW FAR ADVANCED IS THE REPLACEMENT OF ANIMAL EXPERIMENTATION? In Vitro Toxic 1993;6(3):149-161.

The origins of the concept of replacement alternatives in the 1950s, and the impact of societal changes in the 1960s and 1970s, resulting in stricter controls on animal experimentation from the 1980s, based on the Three Rs of Russell and Burch (reduction, refinement and replacement), are reviewed. The range of replacement alternative methods, and some of the ethical issues they raise, and progress toward their incorporation into teaching, fundamental and applied research, and, in particular, toxicity testing, are discussed. Finally, it is concluded that much greater effort should be put into overcoming the barriers to the acceptance of replacement alternatives, which currently limit the contributions they have to make toward greater humanity and better biomedical science. Particular emphasis is placed on the need to ensure that the validation of non-animal tests (for their reproducibility and relevance for specific purposes) is conducted fairly and objectively, and that greater heed is paid to the warning given by Russell and Burch about the high fidelity fallacy and the relevance of

data provided by animal models for human hazard and risk assessment.

248
Fentem J, Balls M. REPLACEMENT OF FISH IN ECOTOXICOLOGY TESTING USE OF BACTERIA OTHER LOWER ORGANISMS AND FISH CELLS IN VITRO. Richardson, M. (ED.). Ecotoxicology Monitoring; International Symposium, London, England, UK, June 17, 1992. XXV+384P. VCH Verlagsgesellschaft MBH: Weinheim, Germany; VCH Publishers, Inc.: New York, New York, USA. ISBN 3-527-28560-1; ISBN 1-56081-736-4. 1993;0(0):71-81.

No abstract.

249
Rowan A, Goldberg A. RESPONSIBLE ANIMAL RESEARCH A RIFF OF R'S. In Vitro Toxic 1993;6(3):141-148.

No abstract.

250
Rowan A, Goldberg A. RESPONSIBLE ANIMAL RESEARCH A RIFF OF R'S. In Vitro Toxicol 1993; 6(3):141-148.

No abstract.

251
Lewtas J, Mumford J, Everson RB, Hulka B, Wilcosky T, Kozumbo W, Thompson C, George M, Dobias L, Sram R, Li X, Gallagher J. COMPARISON OF DNA ADDUCTS FROM EXPOSURE TO COMPLEX MIXTURES IN VARIOUS HUMAN TISSUES AND EXPERIMENTAL SYSTEMS. Environ Health Perspect 1993;99:89-97. (37 REFS)

The results of DNA adduct formation induced by in-vivo and in-vitro exposure to complex environmental mixtures were summarized. DNA adduction induced by cigarette smoke, coke oven emission, smoky coal, diesel exhaust, aluminum smelter, or urban air extracts was studied in-vitro in calf thymus DNA and in-vivo in mouse skin and lung tissue. The level of DNA adduction in white blood cells (WBCs), lymphocytes, placental tissue, lavaged lung cells, sperm, and heart and lung tissue obtained at autopsy from smokers and nonsmokers and in persons exposed to emissions from coke ovens, aluminum

smelters, and smoky coals was measured. The concentrations of DNA adducts were determined by the phosphorus-32 post labeling/thin layer chromatography assay. In calf thymus DNA, the coke oven emission extract was the most potent inducer of DNA adducts followed by the smoky coal, diesel, and cigarette smoke extracts. High levels of DNA adducts were induced in-vivo in mouse skin and lung tissues by the coke oven, smoky coal, aluminum smelter, and urban air extracts. The adduct concentrations were generally higher in the skin and correlated with the incidence of skin tumors. The adducts were chromatographically similar to those induced in calf thymus DNA. A major adduct in lung DNA that cochromatographed with a benzo(a)pyrene derived adduct was induced by all extracts. DNA adduct concentrations were significantly higher in most tissues from smokers than from nonsmokers. The largest difference was observed for heart and lung DNA. Sperm DNA concentrations from smokers and nonsmokers were similar. Coke oven, aluminum smelter, and smoky coal emissions produced higher adduct concentrations in most tissues than cigarette smoke. In-vitro exposure of human lymphocytes, WBCs, and lavaged lung cells to smoky coal extracts induced significant increases in DNA adduction in lymphocytes and lung cells, but not in the WBCs. The authors conclude that persons exposed to complex environmental mixtures have significantly increased levels of DNA adduction. The DNA adduct concentrations are higher in target tissues such as the lung than in nontarget tissues such as lymphocytes and WBCs.

252
Tein I, Dimauro S, Xie ZW, De Vivo DC. VALPROIC ACID IMPAIRS CARNITINE UPTAKE IN CULTURED HUMAN SKIN FIBROBLASTS. AN IN VITRO MODEL FOR THE PATHOGENESIS OF

VALPROIC ACID-ASSOCIATED CARNITINE DEFICIENCY. Pediatr Res 1993; 34(3):281-7.

The mechanisms of valproate-assocd. carnitine deficiency are controversial. The urinary excretion of valproylcarnitine is insufficient to account for tissue carnitine depletion. To explore this mechanism, the authors studied the effects of valproic acid (VPA) on carnitine uptake in cultured human skin fibroblasts by the method of Tein et al.(1990). Fibroblasts were preincubated with varying concns. (0-2000 muM) of VPA for 1, 3, 5, 7, 10, 14, 21, and 28 d and then incubated with fixed carnitine concentration of 50 muM (normal

physiol. concn.), 20 muM (as seen in secondary carnitine deficiency disorders), or 5 muM (as seen in the plasma membrane carnitine transport defect). There was an exponential dose-dependent decrease in carnitine uptake with increasing VPA concentrations, and the relative inhibitory effect was the same for all three carnitine concentrations. The mean percentages .+-. SD (n-1) of residual carnitine uptake for all combined preincubation periods (1-28 d) and combined carnitine concns. (5, 20, and 50 mumol/L) with increasing concentrations of VPA varied from 83.4 .+-. 2.6% (10 muM VPA) to 56.7 .+-. 0.1% (500 muM) to 19.8 .+-. 1.3% (2000 .mu.M). The degree of inhibition was directly proportional to the time of VPA preincubation and parallel for all three carnitine concentrations; the longer the preincubation period, the lower the toxic dose of VPA (to a min. of 450 muM), resulting in a 50% suppression of carnitine uptake (TD50). The mean TD50 of the combined carnitine concentrations for increasing preincubation periods of VPA varied from 1898 .+-. 214 muM (1 d) to 447 .+-. 9 muM (28 d), tapering toward an asymptote of 450 muM when the preincubation period exceeded 14 d. This in vitro TD50 value may be comparable to the in vivo therapeutic range of serum VPA concns. (350-700 mumol/L) for anticonvulsant therapy. The authors conclude that one mechanism by which long-term VPA therapy induces serum and tissue carnitine depletion is through inhibition of plasmalemmal carnitine uptake, including decreased renal reabsorption of free carnitine. This effect is directly proportional to the duration of exposure and concentration of VPA.

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Halmos G, Rekasi Z, Szoke B, Schally AV. USE OF RADIORECEPTOR ASSAY AND CELL SUPERFUSION SYSTEM FOR IN VITRO SCREENING OF ANALOGS OF GROWTH HORMONE-RELEASING HORMONE. Receptor 1993;3(2):87-97.

In the search for more active agonists and antagonist of human growth hormone-releasing hormone (hGH-RH), various analogs are being synthesized. In order to follow the binding affinity of these analogs, the authors have developed a sensitive in vitro radioreceptor assay for GH-RH based on binding of labeled [His1,Nle27]hGH-RH(1-32)NH2 to rat anterior pituitary membrane homogenates by adapting and modifying earlier methods. Scatchard anal. of satn. binding data demonstrated the presence of a single class of specific binding sites for GH-RH in membranes

of rat anterior pituitaries with a Bmax of 33.3 fmol/mg protein and an apparent Kd of 0.19 nM. In displacement analyses, the authors compared the binding affinity of [His1,Nle27]hGH-RH(1-32)NH2 with its iodinated deriv. No significant differences were detected in IC50 concns. ranging from 0.97 to 3.4 nM between labeled and nonlabeled hGH-RH analogs. These findings demonstrate the validity of the radioreceptor assay. To evaluate the biol. activities of hGH-RH derivs., the authors applied a sensitive, dispersed rat pituitary cell superfusion system. This dynamic in vitro system eliminates the drawbacks of the static pituitary cell culture. No differences were obsd. in biol. activities of the iodinated and noniodinated hGH-RH analogs. GH-releasing activity obtained from the superfusion assay correlated well with GH-RH receptor binding affinity for all nonlabeled and labeled hGH-RH analogs examd. These two methods are fast, simple, and relatively inexpensive, and provide quant. data on receptor affinities, biol. activities, and hence structure affinity and structure-activity relationships. Joint use of these two in vitro systems appears to be suitable for screening newly synthesized GH-RH analogs.