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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

Chambers JE, Carr RL. INHIBITION PATTERNS OF BRAIN ACETYLCHOLINESTERASE AND HEPATIC AND PLASMA ALIESTERASES FOLLOWING EXPOSURES TO THREE PHOSPHOROTHIONATE INSECTICIDES AND THEIR OXONS IN RATS. Fundam Appl Toxicol 1993;21(1):111-119.

Rats were administered high sublethal intraperitoneal dosages of the phosphorothionate insecticides parathion, methyl parathion, and chlorpyrifos, and their oxons. Acetylcholinesterase activities in cerebral cortex and medulla oblongata and aliesterase activities in liver and plasma were monitored at 2 hr and 1, 2, and 4 days after exposure. The maximal inhibition of brain acetylcholinesterase activity was not immediate with parathion and chlorpyrifos, reflecting the time required for bioactivation of the phosphorothionates as well as the effectiveness of the aliesterases to inactivate much of the hepatically generated oxons. In contrast, brain acetylcholineasterase activities were more quickly inhibited following administration of paraxon and chlorpyrifos-oxon, which do not require bioactivation. Brain acetylcholinesterase was also rapidly inhibited following administration of methyl parathion and methyl paraoxon, reflecting the low sensitivity of the aliesterases to methyl paraoxon. Aliesterases were inhibited to a greater extent than acetylcholinesterase at each sampling time with parathion and chlorpyrifos and their oxons, whereas the reverse was true with methyl parathion and methyl paraoxon. All of the above patterns correlate with the in vitro sensitivities of acetylcholinesterase and aliesterases to the oxons. The very prolonged inhibition of esterase activities following chlorpyrifos treatment probably results from its substantially greater lipophilicity compared to the other compounds, which would allow it to be stored and released for gradual bioactivation. The data reported indicate that the disposition and effects of different phosphorothionate insecticdes will be influenced by the sensitivities of target and nontarget esterases for their oxons and by their lipophilicity, and that predictions of in vivo responses can be made from in vitro data.

2

Gierthy JF, Swami K, Narang R, Narang A, Eadon G. DETECTION OF DIOXIN-LIKE ACTIVITY IN DIELECTRIC FLUIDS BY AN EPITHELIAL CELL CULTURE BIOASSAY. Chemosphere

1993; 26(6):1225-1235. (REFS 21)

The feasibility of the flat cell assay for detecting the conversion of polychlorinated biphenyls (PCBs) to polychlorinated dibenzofurans (PCDFs) was investigated in dielectric fluids. XBF cells were fed ten fold dilutions of dibenzo-p-dioxins, PCDFs, PCBs, and aroclors. Comparisons were made with the 2,3,7,8-tetra-chlorodibenzo-p-dioxin (1746016) (TCDD) calibration standard with respect to flat cell inducing activity. Results showed that TCDD was the most active compound. Pyrolyzed samples were associated with a 100 fold increase in bioassay activity and increased PCDF concentrations. The testing of six transformer fluids showed that the bioassay is useful for screening for dioxin related aggregate activity. The authors state that this low cost, high capacity bioassay could be used to generate information in risk assessments and could also work as a complement to chemical analyses.

CANCER

3

Saikawa Y, Kubota T, Kuo TH, Kase S, Furukawa T, Tanino H, Ishibiki K, Kitajima M. SYNERGISTIC ANTITUMOR ACTIVITY OF MITOMYCIN C AND CISPLATIN AGAINST GASTRIC CANCER CELLS IN VITRO. J Surg Oncol 1993;54(2):98-102.

The synergistic antitumor activity of mitomycin C (MMC) and cisplatin (DDP) against the gastric cancer cell lines MKN-28 and MKN-45 was assessed in vitro using the MTT assay. The synergism of the two agents was evaluated in terms of the interaction index (I.I.). The sequence of MMC followed by DDP showed higher antitumor activity than the reverse sequence against MKN-28 and MKN-45, and the intracellular concentration of platinum was significantly increased in MKN-45 by preincubation with MMC, suggesting that MMC modulates cellular permeability to DDP or the ability of DDP to intercalate DNA. Since these two antitumor agents show different types of toxicity clinically, i.e., myelotoxicity by MMC and nephrotoxicity by DDP, this combination chemotherapy could be advantageous by providing synergistic antitumor activity without increased toxicity.

4

Bergmann L, Fenchel K, Weidmann E, Enzinger HM, Jahn B, Jonas D, Mitrou PS. DAILY ALTERNATING ADMINISTRATION OF HIGH-DOSE ALPHA-2B-INTERFERON AND INTERLEUKIN-2

BOLUS INFUSION IN METASTATIC RENAL CELL CANCER. A PHASE II STUDY. Cancer 1993; 72(5):1733-42.

Both interleukin-2 (IL-2) and alpha-interferon (alpha-IFN) have some efficacy in renal cell cancer (RCC) as single agents. Additionally, there is some evidence for additive or synergistic antitumoral activity of IL-2 and alpha-IFN in vitro and possibly in vivo. Based on these data, the authors initiated a Phase II trial with a combination of recombinant IL-2 (rIL-2) and recombinant alpha-IFN (alpha-rIFN) in advanced RCC. METHODS. Thirty-six assessable patients with metastatic RCC were entered in this Phase II trial using a daily alternating schedule of alpha-rIFN and rIL-2. Over a period of 14 days, the patients received daily alternating treatment with 10 x 10(6) IU/m2 of recombinant alpha-2b-interferon subcutaneously and 18 x 10(6) IU/m2 of rIL-2 as a 1-hour intravenous infusion. This treatment schedule was repeated every sixth week up to a maximum of four cycles. After the second cycle, patients were examined for response. Patients with stable disease or better received two additional cycles of therapy. Patients with progressive disease were available for other strategies. RESULTS. Thirty-six patients entered the trial and were assessable for toxic effects. Thirty of 36 patients completed at least two cycles and were assessable for response. Nine patients achieved an objective response: 2 had complete responses (CR) and 7 had partial responses (PR). Three patients had a minor response. No effect was observed in patients with local relapse or bone metastases. A relapse-free survival length of 6 months or longer was seen in both patients with CR (12, 23 + months) and in four of seven patients with PR (6, 7, 12, 12 months). The toxicity was moderate and included fever and nausea in most patients, and hypotension, fatigue, skin rash, and arthralgia in a minority of the patients. No Grade 4 and only occasionally Grade 3 toxicity was observed. Fluid retention was negligible. The monitoring of immunologic parameters showed a significant rebound lymphocytosis including cytotoxic (CD56+) cells; in responders the peak of lymphocytosis occurred up to 1 week later than in nonresponders. Peripheral lymphocytes obtained after therapy showed only a slight increase of natural killer cell and lymphokine-activated killer cell activity. During therapy, there was a great release of secondary cytokines as tumor necrosis factor-alpha, gamma-interferon, and interleukin-6, with a peak level 2-4 hours after rIL-2 infusion. CONCLUSIONS. In conclusion, daily alternating administration of

alpha-rIFN and rIL-2 is effective in RCC with less toxicity, and the response rate is comparable to those of other immunotherapeutic schedules, including adoptive immunotherapeutic schedules, including adoptive immunotherapy and combinations of high-dose IL-2 and alpha-IFN.

5
Furukawa T, Kubota T, Matanabe M, Kitajima M, Hoffman RM. A NOVEL "PATIENT-LIKE" TREATMENT MODEL OF HUMAN PANCREATIC CANCER CONSTRUCTED USING ORTHOTOPIC TRANSPLANTATION OF HISTOLOGICALLY INTACT HUMAN TUMOR TISSUE IN NUDE MICE. Cancer Res 1993; 53(13):3070-2.

A new cancer treatment model was developed using the human pancreatic cancer cell line PANC-4 orthotopically transplanted to the pancreas of nude mice as a histol. intact tumor tissue. The tumor grew with subsequent invasive local growth and liver and peritoneal metastases. The antitumor activity of 5-fluorouracil (5-FU) and mitomycin C (MMC) against PANC-4 was initially detd. in vitro in collagen sponge gel-supported tissue culture drug-response assays with the MTT end point. The inhibition rates were 5.6% for 5-FU and 39.4% for MMC, indicating higher efficacy of MMC than 5-FU against PANC-4. When the in vivo antitumor activities of 5-FU and MMC against PANC-4 were determined in the nude mouse model, slight local tumor growth inhibition with equiv. incidence of metastases to the liver and peritoneum as in controls were observed in mice treated with 5-FU, while mice treated with MMC had considerably reduced local tumor growth without liver and peritoneal metastases. Thus, the histoculture drug response assays in combination with orthotopic transplant metastasis models allow evaluation of antitumor agents which may be effective against locally growing human pancreatic cancer and its etastases.

6
Lai T, Collins CM, Hall P, Morgan AP, Smith PJ, Stonebridge BR, Symes MO. VERAPAMIL ENHANCES DOXORUBICIN ACTIVITY IN CULTURED HUMAN RENAL CARCINOMA CELLS. Eur J Cancer 1993;29A(3):378-83.

Cells from 22 renal cell carcinomas (RCC) were established in culture. Sensitivity of the tumour cells to doxorubicin alone and in combination with racemic verapamil, which reverses multidrug resistance, was

tested using a [75Se]selenomethionine uptake assay to measure protein synthesis. The effect of verapamil was expressed as a potentiation index: LD50doxorubicin/ LD50doxorubicin + verapamil. The potentiation index in 15 of these carcinomas was determined for cells within the first 14 days of culture. At 3.3 mumol/l concentration of verapamil, of the tumours sensitive to doxorubicin alone (LD50 < 0.75 microgram/ml) five of seven showed a potentiation index of > 2. For the less sensitive tumours the analogous proportion was seven of eight. Tumour cell expression of glycoprotein P-170, associated with multidrug resistance, was estimated using the monoclonal antibody C-219. Initial expression levels were unrelated to the action of verapamil. In five tumours the proportion of cells expressing P-170 declined as the period of culture increased. This was not associated with any consistent change in the LD50 for doxorubicin or in potentiation of doxorubicin sensitivity by verapamil. Cell cloning associated with prolonged cell growth in vitro could mimic tumour cell cloning which accompanies the formation of metastases. Thus reduced expression of P-170 on prolonged cell growth in vitro may be a pointer to the efficacy of combination therapy in the treatment of patients with metastatic renal cell carcinoma.

CARCINOGENICITY

7

Shepherd JG, Chen JR, Tsao MS, Duguid WP. NEOPLASTIC TRANSFORMATION OF PROPAGABLE CULTURED RAT PANCREATIC DUCT EPITHELIAL CELLS BY AZASERINE AND STREPTOZOTOCIN. Carcinogenesis 1993;14(5):1027-33.

The role of duct cells in the histogenesis of pancreatic carcinoma was studied using a propagable cultured pancreatic duct epithelial cell line derived from a Fischer-344 rat. Tumorigenic transformation was induced by treatment with two experimental pancreatic carcinogens, azaserine and streptozotocin, or spontaneously using a 'selective' culture condition. Tumors arising from spontaneously transformed cells were anaplastic carcinomas, while those from streptozotocin-transformed cells were well or moderately to poorly differentiated adenocarcinomas. Ultrastructural evidence of acinar or endocrine differentiation was absent. The biochem. phenotypes of representative tumor cell lines established from these tumors were studied. As compared to the parental cell line which expressed high activity of carbonic anhydrase (CA) and negligible activity of

gamma-glutamyl transpeptidase (GGT), the tumor cell lines displayed variably increased levels of GGT, and a diminution or loss of CA activity. The tumor cell lines also showed heterogeneity in proto-oncogene and growth factor/receptor expression. The transforming growth factor-alpha mRNA expression was increased in all tumor cell lines, esp. in those induced by azaserine. In contrast, mRNA expression of epidermal growth factor receptor was markedly down-regulated in all tumor cell lines. All chemically induced tumor cell lines showed marked overexpression of the c-myc and c-Ki-ras mRNAs, whereas the spontaneously transformed tumor cell line showed only a significant overexpression of the c-Ki-ras. Point mutation of this proto-oncogene at codons 12, 13 or 61 was absent. The results show that azaserine and streptozotocin are potent carcinogens in vitro for cultured rat pancreatic duct epithelia cells and the phenotype of the tumors is modulated by the method or agent used for their transformation.

8
Dertinger SD, Torous DK, Tometsko AM. IN VITRO SYSTEM FOR DETECTING NONGENOTOXIC CARCINOGENS. Environ Mol Mutagen 1993;21(4):332-338.

Chemical risk assessment has been limited by the inability of in vitro short-term assays to identify the true carcinogenic potential of many substances. Numerous methods exists for identifying mutagenic and clastogenic agents, but a practical means of identifying nongenotoxic carcinogens has remained elusive. Experiments described here suggest that some chemicals may participate in carcinogenesis by modulating the enzymatic processes of drug metabolism. The tumor promoters butylated hydroxyanisole, butylated hydroxytoluene, deoxycholic acid, reserpine, trypan blue, and 12,O-tetradecanoyl phorbol-13-acetate were chosen as model non-genotoxic carcinogens. The enzyme- modulating action of these chemicals was measured using a modified Ames plate incorporation assay whereby the known tumor promoters were plated with a promutagen in the presence of a mammalian metabolic activation system (S9). Each of the nongenotoxic carcinogens significantly increased the mutagenic response of metabolically activated promutagen(s). These experiments suggest that the carcinogenic role of some chemicals may be attributed to their ability to modify the biochemical pathways of drug metabolism. By enhancing or inhibiting the activity of various enzymes, some tumor promoters may

create an environment that increases a cell's mutational burden, thereby contributing to neoplastic transformation.

9
Benigni R, Andreoli C. RODENT CARCINOGENICITY AND TOXICITY, IN VITRO MUTAGENICITY, AND THEIR PHYSICAL CHEMICAL DETERMINANTS. Mutat Res 1993;297(3):281-92.

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.

CELL CULTURE
10
Ohyagi Y, Tabira T. EFFECT OF GROWTH FACTORS AND CYTOKINES ON EXPRESSION OF AMYLOID BETA PROTEIN PRECURSOR MRNAS IN CULTURED NEURAL CELLS. Mol Brain Res 1993; 18(1-2):127-32.

The authors analyzed the effect of several growth factors and cytokines on the expression of amyloid beta protein precursor (APP) mRNAs in cultured mouse neuronal and glial cells. In neuronal cultures from embryonic day-15 brain, Northern blotting revealed that APP mRNAs increased by 1.3-2.6-fold when treated with NGF, basic GFG, interleukin 1, interleukin 2, interleukin 3, interleukin 6 or granulocyte-macrophage colony-stimulating factor but not with tumor necrosis factor .alpha.. An S1 nuclease protection assay revealed that the postnatal day-2 brain did not show any alteration in the presence these factors. The authors conclude that certain growth factors and cytokines could enhance APP 695 mRNA expression in neurons in vitro.

11
Roux H, Duval JL, Sigot-Luizard MF, Sigot M. ASSESSMENT OF THE CYTOCOMPATIBILITY OF BIOMATERIALS BY ANALYSIS OF CELLULAR VIABILITY IN A COULTER MULTISIZER MULTICHANNEL ANALYZER. Adv Biomater 1992;

10(Biomaterial-Tissue Interfaces):81-7.

An in vitro organotypic culture method which allowed cytocompatibility assessment of biomaterials as a function of three biol. properties: cell proliferation, cell migration and cell adhesion was previously described. Another parameter seemed interesting to be studied: cell viability. For this purpose a Coulter Multisizer (Coultronics) provided a programmed technique of analyzing cell size distribution. The percentage of cell viability was calculated by the trypan blue exclusion test. Then an interval which contained a percentage of cells equal to that of cell viability inside the peak of total cells was determined. This method is rapid, easy and give same results as trypan blue exclusion test. It can be spread to different cell types. Moreover the Coulter Multisizer (Coultronics) can be coupled to a computer for the data analysis.

12
Peiser C, Riebe-Imre M, Emura M, Mohr U. INFLUENCE OF CULTURE PASSAGES ON GROWTH KINETICS, XENOBIOTIC METABOLISM, CHROMOSOMAL STABILITY AND TRANSFORMATION IN A CLONAL FETAL HAMSTER LUNG EPITHELIAL CELL LINE. Mutat Res 1993;289(2):281-90.

M3E3/C3 is a clonal fetal hamster lung epithelial cell line which is used for studies of epithelial differentiation as well as for in vitro toxicologic tests. In this study growth kinetics, xenobiotic metabolism, chromosomal stability and transformation were investigated at increasing culture passage numbers up to 150. Cells of higher passages grew faster and reached higher cell densities than the cells of lower ones. As an indicator of xenobiotic metabolism we measured the activity of 7-ethoxycoumarin-deethylase (ECD), an enzyme belonging to the mixed function oxidase system. Up to passage number 100 the ECD activity strongly increased, followed by a slight decrease in additional passages. The chromosomal stability was assessed by the induction of micronuclei by benzo[a]pyrene (BaP) and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). More micronuclei were always detected in cells of higher passages than of lower passages. The capability of cells to be transformed to anchorage independent growth by chemical carcinogens was examined using a soft agar test. After carcinogen exposure with BaP and MNNG, cells of higher passages showed higher transformation frequencies than cells of lower passages. Many cells at passage 150 exhibited an especially high soft agar growth even without carcinogen treatment and were therefore characterized as spontaneously transformed. These results show that metabolic and genetic characteristics of permanently growing cells differ remarkably depending on the culture passage. This has always to be considered when permanently growing cells are used for toxicological studies.

13
Kasson BG, Bai S, Liu J, Tobin C, Kellel B. CHARACTERIZATION OF A RAPID AND SENSITIVE ENZYME IMMUNOASSAY (EIA) FOR PROGESTERONE APPLIED TO CONDITIONED CELL CULTURE MEDIA. J Immunoassay 1993; 14(1-2):33-49.

Progesterone accumulation in conditioned media is a frequently employed endpoint for in vitro cell culture

of steroidogenic cells. A microtiter plate EIA for the detn. of progesterone in conditioned media is characterized. The EIA has a sensitivity of 0.3 pg per well with intraassay and interassay relative std. deviations of 7.3 and 10.2%, resp. The specificity of the EIA is not different from that of a comparable RIA, showing cross reactivities of <0.1% for other steroids except 5alpha- pregnan- 3,20-dione (47%) and 11alpha-hydroxyprogesterone 18%). Progesterone levels from conditioned cell culture media of either rat or human granulosa cell cultures measured by both EIA and RIA were in close agreement, and serial dilns. of

culture samples in the EIA were parallel to those of the stds. Also, extn. of culture media prior to EIA was not necessary. Thus, this EIA is a highly sensitive and specific assay that provides a rapid, simple, inexpensive, and non-radiometric alternative to RIA for measurement of progesterone in conditioned cell culture media.

14
Al-Katib A, Mohammad R, Hamdan M, Mohamed AN, Dan M, Smith MR. PROPAGATION OF WALDENSTROM'S MACROGLOBULINEMIA CELLS IN VITRO AND IN SEVERE COMBINED IMMUNE DEFICIENT MICE: UTILITY AS A PRECLINICAL DRUG SCREENING MODEL. Blood 1993; 81(11):3034-42.

Waldenstroem's macroglobulinemia (WM) represents an indolent incurable human B-cell tumor. The authors have successfully established a permanent cell line, WSU-WM, without growth factors or viral transformation, from the pleural effusion of a 60-yr-old man with IgMkappa WM. Phenotypic characterization of WSU-WM shows IgMlambda and expression of other B-cell markers. Karyotypic analysis shows a male chromosome complement with several clonal aberrations, including t(8;14)(q24;q32). Mol. characterization shows deletion of kappa and rearrangement of lambda light chain genes indicating a class switching. Both the secretory (smu) and membrane (mmu) components of IgM are expressed. In additoin, the breakpoint on 8q24 is downstream of exon 3 of the c-myc oncogene. WSU-WM grows in liq. culture and soft agar. When cells were injected s.c. in immune deficient mice, six of seven SCID mice developed s.c. tumors as opposed to three of seven in the athymic nude mice. When a WSU-WM SCID tumor was passaged in vivo in the SCID mice, the take rate was 100%. This xenograft model and a soft agar disk-diffusion assay were used to test the efficacy of std. chemotherapy agents against

this tumor in vivo and in vitro, resp. The cell line and the assays described herein can be used as a model to facilitate the discovery of new therapeutic agents or modalities for this disease.

15
Mesnil M, Piccoli C, Yamasaki H. AN IMPROVED LONG-TERM CULTURE OF RAT HEPATOCYTES TO DETECT LIVER TUMOR-PROMOTING AGENTS: RESULTS WITH PHENOBARBITAL. Eur J Pharmacol, Environ Toxicol Pharmacol Sect 1993;248(1):59-66.

Among various cocultures of hepatocytes with other cell types, it was found that mouse embryonal cells (BALB/c 3T3) are more effective in maintaining rat hepatocytes in vitro. Because most human cancers are epithelial in origin, it was thought that such a hepatocyte culture system could be used for the detection of tumor-promoting agents, most of which are inhibitors of gap-junctional intercellular communication. Therefore, the authors examined the effect of the strong rat liver tumor promoter, phenobarbital, on the gap-junctional intercellular communication capacity of hepatocytes in long-term cultures. A single application of phenobarbital drastically inhibited the gap-junctional intercellular communication between hepatocytes in a coculture for only several hours, but treatment for 3 wk provoked a const. decrease of gap-junctional intercellular communication (50%) throughout the treatment period. This type of long-term culture of rat hepatocytes may be usable in a rapid in vitro assay to detect tumor-promoting agents.

16
Wells D. THE IN VITRO ISOLATION OF MURINE EMBRYONIC STEM CELLS. Methods Mol Biol (Totowa, N. J.) 1993;18(Transgenesis Techniques):183-216. (REFS 51)

Embryonic stem (ES) cells are derived directly from those progenitor cells of early mouse embryos that subsequently form all of the tissues of the fetus itself and that under appropriate culture conditions, can be maintained continuously in an undifferentiated state in vitro. This review details the materials and methods required for the isolation of murine ES cells; to date the mouse is the only species in which ES cells have been isolated and shown to be pluripotent in vivo. Embryonic cells that morphol. resemble murine ES cells have been isolated from the golden hamster, pig, sheep,

and cow; however, no one has yet demonstrated that these cells are capable of participating in normal embryo development and contributing to a chimeric animal in these species. The subsequent sections describe: the tissue-culture facility required; media and std. culture procedures used; the prodn. of mouse embryos from different development stages; the procedures used for the isolation of ES cells from each of these different embryonic stages; and routine in vitro ES cell-culture techniques.

17
Mullins RD, Sisken JE, Hejase HAN, Sisken BF. DESIGN AND CHARACTERIZATION OF A SYSTEM FOR EXPOSURE OF CULTURED CELLS TO EXTREMELY LOW FREQUENCY ELECTRIC AND MAGNETIC FIELDS OVER A WIDE RANGE OF FIELD STRENGTHS. Bioelectromagnetics 1993; 14(2):173-186. (REFS 13)

A system for exposing cultured cells to extremely low frequency (ELF) electric and magnetic fields was described. The system was able to produce much higher fields than the Helmholtz coils normally used in ELF electric and magnetic bioeffects research. The system produced uniform magnetic field distributions with flux densities from the microtesla range up to 0.14 tesla and induced electric field strengths on the order of 1.0 volts/meter with no possibility of contamination by electrode products. Independent variation of the applied electric and magnetic fields was also possible using this system. The system employed a ferromagnetic core to contain and direct the magnetic field of a 1,000 turn solenoidal coil. Changing the sample chamber configuration without changing the exposure magnetic fields permitted accurate variation of the induced electric field. The system had the ability to separate the bioeffects of magnetic and induced electric fields. The maximum total harmonic distortion of the induced electric field was typically less than 1.0% in the frequency range of 4 to 100 hertz and magnetic flux density range of 0.005 to 0.14 tesla. The sample temperature was constant to within 0.4 degrees-C by constant perfusion of warmed culture medium through the sample chamber.

18
Mesnil M, Piccoli C, Yamasaki H. AN IMPROVED LONG-TERM CULTURE OF RAT HEPATOCYTES TO DETECT LIVER TUMOUR-PROMOTING AGENTS: RESULTS WITH PHENOBARBITAL.

Eur J Pharmacol 1993;248(1):59-66.

Among various cocultures of hepatocytes with other cell types, we found that mouse embryonal cells (BALB/c 3T3) are more effective in maintaining rat hepatocytes in vitro. Because most human cancers are epithelial in origin, we thought that such a hepatocyte culture system could be used for the detection of tumour-promoting agents, most of which are inhibitors of gap-junctional intercellular communication. We, therefore, have examined the effect of the strong rat liver tumour promoter, phenobarbital, on the gap-junctional intercellular communication capacity of hepatocytes in long-term cultures. A single application of phenobarbital drastically inhibited the gap-junctional intercellular communication between hepatocytes in a coculture for only several hours, but treatment for 3 weeks provoked a constant decrease of gap-junctional intercellular communication (50%) throughout the treatment period. This type of long-term culture of rat hepatocytes may be usable in a rapid in vitro assay to detect tumour-promoting agents.

19
Chakradeo PP, Kayal JJ, Bhide SV. EFFECT OF BENZO(A)PYRENE AND METHYL(ACETOXYMETHYL) NITROSAMINE ON THYMIDINE UPTAKE AND INDUCTION OF ARYL HYDROCARBON HYDROXYLASE ACTIVITY IN HUMAN FETAL OESOPHAGEAL CELLS IN CULTURE. Cell Biol Int 1993;17(7):671-676.

Primary cultures of human fetal oesophageal cells were set up and maintained for 45 days. Epithelial cells were the dominant cell type in the culture for the first four weeks. Thereafter, both epithelial cells and fibroblasts were seen with the fibroblasts becoming the dominant cell type by the 6th week and until the cultures degenerated. The tritiated thymidine uptake showed an upward trend from: day 10 up to day 30, with peak uptake at day 30 in the untreated, B(a)P treated and OAc treated cultures and decreased thereafter. The thymidine uptake levels were significantly higher in the B(a)P treated cultures when compared with levels in the untreated cultures. A concurrent increase/decrease was also seen in the cell number in all the three groups of cultures. Cultures with B(a)P and DMN-OAc showed significantly higher AHH levels as compared with untreated cultures. These results indicate that the human fetal oesophageal cells could be viably maintained under in vitro conditions for long periods of time and also showed capacity to metabolise the

carcinogens through aryl hydrocarbon hydroxylase activity.

20
Grando SA, Cabrera R, Hostager BS, Bigliardi PL, Blake JS, Herron MJ, Dahl MV, Nelson RD. COMPUTERIZED MICROASSAY OF KERATINOCYTE CELL-PLASTIC ATTACHMENT AND PROLIFERATION FOR ASSESSING NET STIMULATORY, INHIBITORY AND TOXIC EFFECTS OF COMPOUNDS ON NONIMMORTALIZED CELL LINES. Skin Pharmacol 1993;6(2):135-147.

Testing of pharmacological agents that affect growth of epidermal keratinocytes (EK) requires a standardized assay. We have developed an assay measuring net effects of stimulatory (e.g. growth factors), inhibitory (e.g. methotrexate) or toxic (e.g. Triton X-100) compounds. The amount of crystal violet staining viable EK attached to the wells of standard 96-well microplates is measured in situ using an ELISA plate reader. Optical density readings are directly converted into cell counts by computer software. Counts obtained by this method strongly correlate with the results obtained using the (3H)thymidine uptake assay and direct cell counts. The assay standardizes measurements of nonimmortalized EK lines with different innate proliferative properties and allows accurate quantitation of EK numbers in the range of 2,500-500,000 EK/well.

21
Kirkpatrick CJ, Dekker A, Doherty PJ, et al. QUANTITATIVE EVALUATION OF CELL INTERACTION WITH BIOMATERIALS IN-VITRO. Advances in Biomaterials, Vol. 10. Biomaterial-Tissue Interfaces; Ninth European Conference on Biomaterials, Chester, England, UK, September 9-11, 1991. XII+533P. Elsevier Science Publishers B.V.: Amsterdam, Netherlands; New York, New York, USA. ISBN 0-444-89065-3.; 0 (0) 1992. 31-41.

No abstract.

22
Markiewicz L, Garey J, Adlercreutz H, Gurpide E. IN VITRO BIOASSAYS OF NON-STEROIDAL PHYTOESTROGENS. J Steroid Biochem Mol Biol 1993;45(5):399-405.

Some of the isoflavonoids present in human diet as well as in urine are expected to exert biologic effects as

they have been reported to bind to estrogen receptors and to be estrogenic in other species. This report describes the in vitro assessment of estrogenic effects of isoflavonoids using human endometrial cells and tissue. The relative estrogenic potencies (EC50 values) of estradiol, 3 dietary isoflavonoids (coumestrol, genistein and daidzein) and one of their metabolites (equol), were estimated by using a recently developed multiwell plate in vitro bioassay based on the estrogen-specific enhancement of alkaline phosphatase (AlkP) activity in human endometrial adenocarcinoma cells of the Ishikawa-Var I line. The maximal AlkP activity elicited by the isoflavonoids tested was a high as that achieved with estradiol and their effects were suppressed by the antiestrogens 4-hydroxytamoxifen and ICI 164,384. These results indicate that estradiol and the isoflavonoids exert their effects on AlkP by similar interactions with the estrogen receptor, with potencies depending on binding affinities. The estrogenic effect on equol was confirmed by another in vitro bioassay, based on the estrogen-stimulated enhancement of prostaglandin F1alpha output by fragments of human secretory endometrium.

23
Maier P, Schwalder H. PHYSIOLOGICAL OXYGEN TENSION MODULATES THE CHEMICALLY INDUCED MITOGENIC RESPONSE OF CULTURED RAT HEPATOCYTES. J Cell Physiol 1993; 156(1):119-129.

Freshly isolated rat hepatocytes were cultured at periportal- (13% O2) or perivenous-like (4% O2) oxygen tension and exposed to subtoxic exposure levels of cyproterone acetate (CPA: 10-330 muM), phenobarbital (PB: 0.75-6 mM), and dimethylsulfoxide (DMSO: 0.1-3.3%) from 24-72 h after seeding. Induced alterations in ploidy, in the number of S-phase cells, the degree of binuclearity, and cellular protein content were determined by twin parameter protein/DNA flow cytometry analysis of intact cells and isolated nuclei. CPA and PB increased whereas DMSO decreased dose dependently the total number of 5-phase cells. The changes differed within individual ploidy classes and were modulated by the oxygen tension. CPA increased and DMSO decreased the number of S-phase cells preferentially among the diploid hepatocytes at periportal-like oxygen tension. In contrast, PB increased binuclearity and S-phase cells mainly among the tetraploid hepatocytes at perivenous-like oxygen tension. Cellular protein content increased dose dependently after exposure to

the hepatomitogens (CPA,PB) and decreased after exposure to DMSO at both oxygen tensions. Comparison with in vitro data proves that chemicals which interact with cells from the progenitor liver compartment (CPA, DMSO) exert their mitogenic activity best in cultur es at periportal-like oxygen tension preferentially in diploid hepatocytes, whereas chemicals which affect cells from the functional compartment show a higher activity at perivenous-like oxygen tension. Physiological oxygen tension seems to be an effective modulator of the proliferative response of cultured rat hepatocytes similar to that expected for periportally or perivenously derived hepatocytes.

24
Kefalas V, Stacey NH. USE OF PRIMARY CULTURES OF RAT HEPATOCYTES TO STUDY INTERACTIVE TOXICITY: CARBON TETRACHLORIDE AND TRICHLOROETHYLENE. Toxicol in vitro 1993; 7(3):235-240.

The use of primary cultures of rat hepatocytes as an experimental model for interactive toxicity studies was evaluated by studying carbon tetrachloride (CCl4) and trichloroethylene (TRI). TRI is known to potentiate CCl4-induced hepatotoxicity in vivo as well as in vitro utilizing rat hepatocyte suspensions and plasma membranes. When hepatocytes were cultured in a sealed system for 48 hr, the presence of TRI and CCl4 induced enzyme leakage, lipid peroxidation and an increase in lactate to pyruvate ratios significantly greater than seen in response to each halocarbon alone. Singly administered CCl4 produced a significant elevation in lactate dehydrogenase while other markers remained statistically unaffected CCl4 toxicity was potentiated in the presence of TRI. Ureogenesis remained unaltered in cultures after exposure to CCl4 and TRI whether dosed singly or in combination. The positive interaction in toxicity and peroxidation of lipids is consistent with the findings in short-term in vitro systems as well as in vivo, thus indicating the suitability of primary cultures of rat hepatocytes for such studies.

Cytotoxicity
25
Xie K, Harvey AL. EVALUATION OF NERVE CELL TOXICITY IN VITRO BY ELECTROPHYSIOLOGICAL AND BIOCHEMICAL METHODS. Toxicol in Vitro 1993;7(3):275-9.

The acute toxicity of the first nine std. chems. in Multicenter Evaluation of In vitro Cytotoxicity Tests (MEIC) program was evaluated in the NG108-15 neuroblastoma cell line by monitoring changes in cell resting membrane potential (RMP) and by using the MTT assay to measure cell viability. Cells were differentiated with dibutyryl-cAMP and then exposed to different concns. of the chems. for 1 h or 24 h. At each concn. and time point, RMPs were measured from about 30 differentiated cells and MTT assays were performed on parallel cultures. IC50 values were obtained from linear regression anal. The results showed that the IC50s from MTT assays correlated closely with those from RMP measurements (r = 0.983 for 1 h exposure; r = 0.933 for 24 h exposure). IC50s of amitriptyline and diazepam were 0.1-1.9 mM; alc. compounds (isopropanol, ethylene glycol, ethanol and methanol) had IC50s from 121.5 mM to 3731.9 mM; paracetamol, aspirin and ferrous sulfate had intermediate cytotoxicity (IC50 2.6-53.5 mM). IC50s decreased markedly with increased exposure time. RMP is expected to be a sensitive indicator of the health of nerve cells; however, its measurement in a large number of cells is laborious. MTT assays are rapid, and the close correlation between IC50s in the two types of assay suggests that MTT assays could be used to evaluate cytotoxicity in neuronal cells in vitro.

26
Chung YT, Park ST, Choi MK, Kim JJ, Mun YJ, Woo WH, Han DS, Choi BK, Soh JT. A STUDY ON THE CYTOTOXICITY OF CADMIUM IN VITRO. Korean J Toxicol 1993;9(1):45-60.

The present study was carried out to evaluate the cytotoxicity of Cd on cultured rat fibroblasts. The colorimetric assays of neutral red (NR) and tetrazolium MTT, the lactate dehydrogenase activity, the amts. of total protein, the rate of DNA synthesis, the amounts of unscheduled DNA synthesis, the frequency of sister chromatid exchange, the releasing rate of intracellular Ca, and light and electron microscopic studies were performed on cultured rat fibroblasts maintained in the media contg. various concns. of Cd. The results were as follows: NR and MTT values were decreased dose-dependently by Cd, and the NR90, NR50, MTT90 and MTT50 values of Cd were 0.2, 21.5, 1.0, and 60.0 muM, resp. Lactate dehydrogenase activity at 10 and 50 muM concns. of Cd were increased to 139 and 282%, resp., compared with control (100%), and the total amts. of protein were decreased dose-dependently by Cd. The rate

of DNA synthesis was decreased dose-dependently by Cd, and the rates of DNA synthesis at 7.0 and 60.0 muM Cd were 90 and 50% of the control, resp. Amts. of unscheduled DNA synthesis and the frequency of sister chromatid exchange were not increased by Cd, however the releasing rate of intracellular Ca was markedly decreased by Cd, compared to the control. In light microscopy, Cd induced the redn. of cells in number and the decrease in cell size dose-dependently, and electron microscopy of cultured fibroblasts at NR50 value showed cisternal dilation of rough endoplasmic reticulum, dense body and numerous vacuoles. Thus, the cytotoxic target of Cd may be cell organelles rather than genetic materials, since a lower concentration of Cd induces more cytotoxic effect on lysosome and mitochondria than DNA.

27
Giridhar J, Acosta D. EVALUATION OF CYTOTOXICITY POTENTIAL OF SURFACTANTS USING PRIMARY RAT KERATINOCYTE CULTURE AS AN IN VITRO CUTANEOUS MODEL. In Vitro Toxicology. A Journal of Molecular and Cellular Toxicology;6(1):33-46. (REFS 23)

The cytotoxicity of several surfactants was studied in rat keratinocyte cultures. Keratinocytes from the skin epidermis of neonatal rat pups were cultured and exposed to octoxynol (Triton-X-100), sodium-lauryl-ethoxy-sulfate (Sipon-ES-7), sodium-cocyl-isothionate (Jordapon-CI-50-Dispersion), lauroamphodiacetate (Miranol-HM), or cocamidopropyl-hydroxysultaine (Mirataine-CBS) for 1 or 24 hours. Cytotoxicity was evaluated by measuring the leakage of lactate-dehydrogenase (LDH), the mitochondrial reduction of 3-(4-5-dimethythiazol-2yl)-2,5-diphenyl-tetrazolium-bromide (MTT), and the lysosomal uptake of neutral-red-dye. A dose dependent leakage of LDH from the cells was seen 1 hour after treatment with each of the surfactants. The release of LDH was increased only two fold over that seen in controls 24 hours after incubation with all of the surfactants with the exception of Triton-X which caused a sustained effect on LDH leakage. The ranked order of the potential to cause cell injury as determined by the LDH and MTT assays was Triton-X, followed by Sipon, Jordapon, Mirataine, and Miranol. Based upon the NR assay, the order was Sipon followed by Jordapon, Triton-X, Mirataine, and Miranol. Overall, the potential to cause cell damage was determined to be equal between the nonionic and anionic surfactants with

the amphoterics having less cytotoxic potential. The authors conclude that cultures of rat keratinocytes are adequate in-vivo models for screening surfactants for skin irritancy potential.

28
Boue-Grabot M, Halaviat B, Pinon JF. SIMPLE METHOD FOR CYTOTOXICITY STUDIES OF NON-HYDROSOLUBLE SUBSTANCES: POSSIBLE APPLICATION AS AN ALTERNATIVE TO THE DRAIZE TEST FOR COSMETICS AND TOILETRIES. Parfuem Kosmet 1993;74(Apr):228-232. (REFS 10)

A method that uses a microporous membrane between a cell culture and a non-aqueous substance for cytotoxicity testing is presented and evaluated on 50 commonly used cosmetic and toiletries products; results were compared with those of the Draize irritation test. Results indicated that there was a good correlation between the calculated cytotoxicity index and the ocular irritation index. It was concluded that this method may provide an alternative to animal studies on the ocular tolerance of cosmetics and toiletries.

29
Hahn SM, Liebmann JE, Cook J, Fisher J, Goldspiel B, Venzon D, Mitchell JB, Kaufman D. TAXOL IN COMBINATION WITH DOXORUBICIN OR ETOPOSIDE. POSSIBLE ANTAGONISM IN VITRO. Cancer 1993; 2(9):2705-11.

Taxol is a novel chemotherapeutic agent that promotes microtubule assembly and stabilizes tubulin polymer formation. Clinical evaluation of its antineoplastic activity as a single agent and in combination with other chemotherapeutic drugs is in progress. METHODS. To evaluate the effect of combining taxol with other commonly used antineoplastic agents, clonogenic survival of human breast cancer MCF7 cells, human lung adenocarcinoma A549 cells, and human ovarian cancer OVG1 cells were assayed after an initial exposure to taxol for 24 hours (approximately LD90 for taxol), followed by a 1-hour incubation with varying concentrations of doxorubicin or etoposide (total taxol incubation time, 25 hours). RESULTS. When corrected for taxol-induced cytotoxicity, doxorubicin and etoposide caused less cell killing in the presence of taxol compared with control incubations of doxorubicin and etoposide alone. To determine if a different schedule of drug application resulted in a similar finding, MCF7, A549, and OVG1 cells were exposed to doxorubicin

for 1 hour, followed by incubation with varying concentrations of taxol for 24 hours. Less-than-additive cytotoxicity for the combination of taxol and doxorubicin was found. Flow cytometry studies in MCF7 cells showed that taxol caused a G2/M cell cycle block. Fewer cells were found to be in S-phase, which is the most doxorubicin-sensitive phase of the cell cycle. The application of doxorubicin or etoposide to MCF7 cells for 1 hour resulted in partial G1 and G2/M cell cycle blocks. Fewer cells were found to be moving through the cell cycle, which is likely required for taxol cytotoxicity. CONCLUSION. Although direct antagonism of the cytotoxicity of doxorubicin or etoposide by taxol has not been proven, there is less-than-additive in vitro cytotoxicity when taxol is combined with these chemotherapeutic agents. The clinical implications of these findings are unknown; however, these findings generate concern about the combination of these agents in clinical trials and suggest that additional studies to determine optimal scheduling are needed.

30
Daoud SS, Sakata MK. IN VITRO INTERACTION OF LIPOSOMAL VALINOMYCIN AND PLATINUM ANALOGS: CYTOTOXIC AND CYTOKINETIC EFFECTS. Anticancer Drugs 1993;4(4):479-86.

Cisplatin is the most active agent in the chemotherapy of ovarian cancer and this activity can be enhanced by liposomal valinomycin (MLV-VM) in vitro. To test whether MLV-VM is capable of augmenting the cytotoxic and cytokinetic effects of other platinum analogs, drug combinations of MLV-VM and platinum drugs were tested against two human ovarian cancer cell lines (OVCAR-3 and CaOV-3) and on Chinese hamster ovary (CHO) cells in vitro. MLV-VM enhanced the sensitivity to cisplatin, ormaplatin and carboplatin on human ovarian carcinoma cells that show various degrees of drug sensitivity. This interaction was shown to be truly synergistic by median-effect analysis up to 90% cell kill. The combination index at 50% cell kill (Cl50) was also used to quantitate the extent of drug synergy. In the OVCAR-3 cell line, for example, the Cl50s were 0.62, 0.85 and 0.8 for cisplatoin, ormaplatin and carboplatin, respectively. DNA histograms btained by flow cytometry showed that CHO cells treated with cisplatin alone accumulated in the S-G2 segment, with a partial G2 block. The addition of 2 microM VM with cisplatin, significantly enhanced the accumulation of cells at the G2/M phase. Our results further demonstrate that in vitro treatment with VM,

cisplatin and/or combination is associated with an increase in protein kinase C (PKC) activity. These findings suggest that accumulation of cells at G2/M phases and modulation of PKC activity could be among the basis for the cytotoxic synergism observed between cisplatin and VM.

31
Lewis RW, McCall JC, Botham PA. A COMPARISON OF TWO CYTOTOXICITY TESTS FOR PREDICTING THE OCULAR IRRITANCY OF SURFACTANTS. Toxicol in Vitro 1993;7(2):155-8.

The aim of the present studies was to compare the predictive ability of 2 in vitro cytotoxicity tests (the K562 and the red blood cell lysis tests) in the assessment of the in vivo eye irritancy of surfactants. The results of these studies on 14 selected surfactant materials showed that the K562 assay was less likely to over-est. effect (when assessed over a wide range of surfactant concn.) than had previously been observed. This increased specificity (86%) was accompanied by a decrease in sensitivity (57%), the ability to correctly identify irritant surfactants. The red blood cell lysis test was more predictive, correctly identifying all irritants tested. In addition, all non-irritant surfactants examd. were predicted by this test and a high (89%) ability to rank irritant effect was demonstrated. The red blood cell lysis test could be a powerful addn. to a testing strategy or pre-screen for the evaluation of surfactant chemicals.

32
Teepe RG C, Koebrugge EJ, Lowik CW G, Petit PL C, Bosboom RW, Twiss IM, Boxma H, Vermeer BJ, Ponec M. CYTOTOXIC EFFECTS OF TOPICAL ANTIMICROBIAL AND ANTISEPTIC AGENTS ON HUMAN KERATINOCYTES IN VITRO. J Trauma 1993;35(1):8-19.

The cytotoxicity of commonly used antimicrobial and antiseptic agents to cultured human keratinocytes was investigated, combining a morphol. assay with a quant. neutral red (NR) spectrophotometric assay. Main outcome criteria of response were the initial cytotoxicity (NR 90) and midpoint cytotoxicity (NR 50) and the highest tolerated dose (HTD), a concn. causing the first obsd. morphol. alterations. These values were compared with commonly administered clin. doses or calculated doses. Thirty-five agents were evaluated; 5 agents were also subjected to an assay for cell

morphol. alterations, and HTD and percentage of cells taken up trypan blue and annihilation dose were assessed. These studies show that in clinical concns. many, but not all, agents exert profound cytotoxic effects. The results suggest that under certain conditions cultured epithelial grafts may be exposed to clinical concns. of neomycin, clindamycin, framycetin, erythromycin, gentamicin, and 0.1% solns. of povidone-iodine. Tetrachlorodecaoxygen anion complex (TCDO) should be further evaluated as a likely effective cleansing agent before culture grafting.

33
Zhang X-X, Chakrabarti S, Malick AM, Richer C-L.
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):213-218.

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 containing 10% fetal calf serum, 0.1% garamycine, 1% glutamine and 1%

phytohaemagglutinin. Styrene-7, 8-oxide (SO), styrene glycol (SG), phenylglyoxylic acid (PGA), S-(1,2-phenyl-2-hydroxyethyl)-glutathione (PEG) (a glutathione conjugate of styrene oxide), N-acetyl-S-(1,2-phenyl-2-hydroxyethyl)-cysteine (NAPEC) in dimethyl sulfoxide (DMSO) were injected into the cultures 36 h after initial culture, so that the exposure time for these metabolites was 36 h. The final concentration of SO was 100 muM and those of the other metabolites were 500 muM. 24 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 derivative 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 possesses some cytogenotoxic potential and hence, these two metabolites together could contribute to the genotoxicity of styrene in human blood lymphocytes.

34
Terse PS, Madhyastha MS, Zurovac O, Stringfellow D, Marquardt RR, Kemppainen BW. COMPARISON OF IN VITRO AND IN VIVO BIOLOGICAL ACTIVITY OF MYCOTOXINS. Toxicon 1993;31(7):913-9.

In vitro assays developed to screen the cytotoxic activity of chemicals in murine (NIH/3T3) and bovine (BE 12-6) embryonic cells were used to determine the concentrations (microgram/ml) of mycotoxins which caused 50% lethality (LC50). Embryonic cells were seeded in 96 well plates, cultured for 72 hr with dilutions of each individual and combinations of mycotoxins, and stained and counted. Verrucarin A and roridin A had the strongest cytotoxic activity, and ergotamine tartrate was least toxic. Furthermore, results correlated with published values of in vivo activity, indicating this assay can be used for acute toxicity screening of compounds.

35
Betti C, Barale R, Pool-Zobel BL. COMPARATIVE STUDIES ON CYTOTOXIC AND GENOTOXIC EFFECTS OF TWO ORGANIC MERCURY COMPOUNDS IN LYMPHOCYTES AND GASTRIC MUCOSA CELLS OF SPRAGUE-DAWLEY RATS. Environ Mol Mutagen 1993;22(3):172-80.

Human lymphocytes (HL) as well as lymphocytes (RL), hepatocytes (RH), and gastric mucosa cells (GM) of Sprague-Dawley rats were treated in vitro for 1 h with methylmercury chloride (MMC, 0.5-4 micrograms/ml) and dimethylmercury (DMM, 5-40 micrograms/ml). The cytotoxicity of the two organic mercury compounds was assessed by dye exclusion, and the extent of induced DNA fragmentation was measured with a single-cell microgel electrophoresis assay. Both MMC and DMM induced DNA damage and cytotoxicity in a dose-related manner in HL, RL, and GM. MMC was more effective in causing a significant increase in median DNA migration than DMM at doses yielding approximately the same degree of cytotoxicity. In rat hepatocytes the MMC-induced DNA damage was, however, lower than in the other cells. An analysis of repair kinetics following exposure to 2 micrograms/ml MMC was carried out in human lymphocytes obtained from an adult male donor. The bulk of DNA repair occurred 90 min after in vitro exposure, and it was about complete by 120 min following cessation of exposure. Finally, in order to

have a basis for extrapolating to the human situation, in vivo studies were performed with Sprague-Dawley rats, also assessing the DNA damage and cytotoxicity in the lymphocytes and gastric mucosa cells. These in vivo results after oral exposure may be directly compared to the in vitro data obtained in the same cells.

36
Xie K, Harvey AL. EVALUATION OF NERVE CELL TOXICITY IN VITRO BY ELECTRO-PHYSIOLOGICAL AND BIOCHEMICAL METHODS. Toxicol in Vitro 1993;7(3):275-279.

The acute toxicity of the first nine standard chemicals in the Multicentre Evaluation of In Vitro Cytotoxicity Test (MEIC) programme was evaluated in the NG108-15 neuroblastoma cell line by monitoring changes in cell resting membrane potential (RMP) and by using the MTT assay to measure cell viability. Cells were differentiated with dibutyryl-cAMP and then exposed to different concentrations of the chemicals for 1 hr or 24 hr. At each concentration and time point, RMPs were

measured from about 30 differentiated cells and MTT assays were performed on parallel cultures. IC50 values were obtained from linear regression analysis. The results showed that the IC50s from MTT assays correlated closely with those from RMP measurements ($r = 0.983$ for 1 hr exposure; $r = 0.933$ for 24 hr exposure). IC50s of amitriptyline and diazepam were 0.1-1.9 mM; alcholic compounds (isopropanol, ethylene glycol, ethanol and methanol) had IC50s from 121.5 mM to 3731.9 mM; paracetamol, aspirin and ferrous sulphate had intermediate cytotoxicity (IC50 2.6-53.5 mM). IC50s decreased markedly with increased exposure time. RMP is expected to be a sensitive indicator of the health of nerve cells; however, its measurement in a large number of cells is laborious. MTT assays are rapid, and the close correlation between IC50s in the two types of assay suggests that MTT assays could be used to evaluate cytotoxicity in neuronal cells in vitro.

37
Cascorbi I, Bittrich H, Ricklinkat J, Voss W, Seyfarth A, Foret M. EFFECTS OF A HETEROGENEOUS SET OF XENOBIOTICS ON GROWTH AND PLASMA MEMBRANES OF MAMMALIAN AND FUNGAL CELL CULTURES. Ecotoxicol Environ Safety 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 addition, 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 log 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 estimation of basic toxicity and the detection of outliers thereby reducing the number of tests with higher animals.

38
Viau CJ, Curren RD, Wallace K. CYTOTOXICITY OF TACRINE AND VELNACRINE METABOLITES IN CULTURED RAT, DOG AND HUMAN HEPATOCYTES. Drug Chem Toxicol 1993;16(3):227-39.

Clinical trials with tacrine (THA) and its principal (1-OH) metabolite (velnacrine) for the treatment of Alzheimer's disease have been hampered by adverse hepatic events that were undetected in preclinical studies. As part of integrated in vivo/in vitro efforts to characterize the role of metabolites in these events, cultured cells were evaluated for their suitability for further mechanistic studies. The relative cytotoxic potentials of THA, three monohydroxy metabolites of THA (including velnacrine, a racemate), the two velnacrine enantiomers, and several known and suspected dihydroxy velnacrine metabolites were determined. Cytotoxicity was evaluated in 24-hour cultures by morphology and by the Neutral Red Uptake Assay. All test articles were evaluated in primary rat hepatocytes and in a human hepatoma cell line (HepG2). THA and velnacrine were also tested in a rat hepatoma cell line (H4) and in primary dog hepatocytes. The metabolic competency of each cell type was determined. Sensitivity to THA and velnacrine was greatest in H4

cells, followed by primary rat and HepG2 cells; dog cells were least sensitive. In HepG2 cells, THA was clearly more cytotoxic (LC50:54 micrograms/ml) than its monohydroxy metabolites (LC50 values: 84 to 190 micrograms/ml); dihydroxy velnacrine metabolites were the least cytotoxic (LC50 values:251 to 434 micrograms/ml); the relative order was comparable in primary rat hepatocytes. Roles for reactive metabolites and/or altered metabolic capabilities of Alzheimer's patients are suggested.

Dermal Toxicity

39

de Lange J, van Eck P, Elliot GR, de Kort W LAM, Wolthuis OL. THE ISOLATED BLOOD-PERFUSED PIG EAR: AN INEXPENSIVE AND ANIMAL-SAVING MODEL FOR SKIN PENETRATION STUDIES. J Pharmacol Toxicol Meth 1992;27(2):71-77.

To overcome most of the disadvantages of current models to investigate percutaneous penetration of drugs or toxic substances, a model is proposed here based on the isolated pig ear, which is obtained at the slaughterhouse, and perfused with oxygenated blood from the same pig. To determine the viability of the preparations, we measured glucose consumption and lactate production as metabolic parameters, Na+ and K+ ions, as well as lactate dehydrogenase activity in blood as markers for cell damage, whereas vasomotor reactivity was assessed by administering noradrenalin and isoxsuprine. After 60 minutes of equilibration, only insignificant changes in these parameters were observed during the subsequent 3-hour test period (longer periods were not tested). A slight weight increase was noted during the total period of 4-hours, presumably due to slight weight edema formation. On the basis of several types of measurements, such as in vivo blood flow and ear temperature and in vitro glucose metabolism, standard procedures were developed. It is concluded that this technique offers an easy to handle, cost-efficient, and animal-saving model for skin penetration studies that lacks most of the disadvantages of existing models.

40

Timmins GS, Davies MJ. FREE RADICAL FORMATION IN ISOLATED MURINE KERATINOCYTES TREATED WITH ORGANIC PEROXIDES AND ITS MODULATION BY ANTIOXIDANTS. Carcinogenesis (OXF) 1993;14(8):1615-1620.

Electron paramagnetic resonance spin-trapping has been used to study the production of free radicals from tert-butyl hydroperoxide, tert-butyl peroxybenzoate, cumene hydroperoxide and ethyl hydroperoxide in isolated murine keratinocytes. Free radical species could be trapped from keratinocytes treated with all peroxides, with radicals produced from both one-electron oxidative and reductive pathways. The hindered phenolic antioxidants butylated hydroxyanisole (BRA) and butylated hydroxytoluene (BHT), which are known to inhibit peroxide-induced tumour promotion in vivo, decreased the amount of radical adduct production at a concentration of 10 mM, with BHA being significantly more effective than BHT. That all the peroxides in this study produced free radicals in keratinocytes, and that BHA and BHT decreased the amounts of radicals trapped, suggests that free radical production by organic peroxide compounds is involved in their in vivo tumour-promoting activity.

41
Harvell JD, Gordon V, Maibach HI. USE OF THE SKINTEX HIGH SENSITIVITY ASSAY FOR PREDICTIVE ASSESSMENTS OF CUTANEOUS FATTY ACID IRRITANCY IN MAN. In Vitro Toxicol 1993;6(2):91-96.

We have tested the irritancy of fatty acids of chain lengths C3--C18 in an in vitro dermal irritancy assay--the Skintex High Sensitivity Assay. The results of a modified in vitro protocol (which more closely mimicked the in vivo protocol) were compared to previously published human data (Stillman, et al., 1975). The previously published in vivo data set represented an example of cumulative (chronic) irritancy (i.e. several repeat applications were needed to produce an irritant effect in vivo). Comparing the in vivo results to the in vitro results, the following parameters for this data set were calculated: sensitivity 83%; specificity 50%; positive predictive value 77%; and negative predictive value 60%. These moderate results may reflect either (1) a difficulty of the Skintex system to accurately predict the results of cumulative irritancy, or (2) the difficulty in defining a human irritant/nonirritant cutoff point, which influences these measures. For example, when the human in vivo irritancy cutoff is disignated a 2, instead of a 1, the sensitivity becomes 100%, specificity 50%, positive predictive value 62%, and negative predictive value 100%.

42
Franz TJ, Lehman PA, Franz SF, North-Root H, Demetrulias JL, Kelling CK, Moloney SJ, Gettings SD. PERCUTANEOUS PENETRATION OF N-NITROSODIETHANOLAMINE THROUGH HUMAN SKIN (IN VITRO): COMPARISON OF FINITE AND INFINITE DOSE APPLICATIONS FROM COSMETIC VEHICLES. Fundam Appl Toxicol 1993;21(2):213-221.

N-Nitroso compounds (nitrosamines) have been detected at the parts per billion level in a wide variety of matrices including industrial chemicals, pharmaceuticals, and food. Although Nnitrosodiethanolamine (NDELA) may be detected as an impurity in some cosmetic products, studies on NDELA absorption through human skin have been limited. A study to determine the extent of NDELA absorption following topical application was therefore undertaken to assist in the proper assessment of risk following unintended exposure. NDELA absorption was measured in vitro through human cadaver skin using isopropyl myristate (IPM) and generic prototype personal-care formulations (sunscreen and shampoo) spiked with (14C)NDELA. When applied as a finite dose at a concentration of 0.06% or lower, NDELA absorption was found to be a linear function of concentration. Total absorption at 48 hr ranged from approximately 35 to 65% of the dose and was formulation dependent (IPM > shampoo:sunscreen). Absorption occurred relatively rapidly from all formulations and peak rates of absorption were seen within the first 5 hr from the IPM and shampoo formulations. When applied as an infinite dose, total NDELA absorption followed a different rank order (shampoo:IPM > sunscreen) and evidence of barrier damage was seen with the shampoo formulation.

43
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 barrier 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.

44
Frantz SW, Beskitt JL, Tallant MJ, Futrell JW, Ballantyne B. GLUTARALDEHYDE: SPECIES COMPARISONS OF IN VITRO SKIN PENETRATION. J Toxicol, Cutaneous Ocul Toxicol 1993;12(4):355-67.

It has been reported that the major portion of an applied dose of glutaraldehyde was recovered from skin at the application site in previous in vivo pharmacokinetic studies with 14C-labeled glutaraldehyde in rats and rabbits. To investigate this finding further, and to compare penetration of glutaraldehyde through human skin with data for animal skin preparations, the potential for in vitro skin penetration of [1,5-14C]glutaraldehyde 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 aq. glutaraldehyde concns. of 0.75% and 7.5% used in the previous in vivo 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% aq. soln. Avs. of <0.5% of the applied radioactivity for the 0.75% soln. and <0.7% for the 7.5% soln. were recovered in the effluents for all the animal species (range of 0.05

[female rats] to 1.73 [male mouse] for the 0.75% soln. and 0.08 [male rat] to 1.44 [female rabbits] for the 7.5% soln.). For human female skin, the recovery was approx. 0.2% for both glutaraldehyde concns. Under these in vitro conditions, glutaraldehyde did not penetrate human breast skin to any substantial degree, and this was largely due to a substantial portion of the dose's binding to the skin during uptake. These results are consistent with previous reports and suggest that only a minimal amt. of glutaraldehyde may be available for systemic uptake and distribution following cutaneous exposure. The potential for absorption may be less for humans than for common laboratory test species.

45
Whittle E, Basketter DA. THE IN VITRO SKIN CORROSIVITY TEST. COMPARISON OF IN VITRO HUMAN SKIN WITH IN VIVO DATA. Toxicol in Vitro 1993;7(3):269-73.

Regulations require that many substances and chems. are tested for their cutaneous toxicity potential. At present this is assessed in an in vivo rabbit dermal test. A rapid, robust and reliable alternative for testing the corrosive potential of substances is the in vitro skin corrosivity test, which alleviates the need to assess corrosive substances in an in vivo test. The method is based on the observation that corrosive substances cause a significant redn. in the elec. resistance of skin. It was anticipated that the more resistant nature of human skin would lead to a no. of substances being classified as non-corrosive, contrary to historical in vivo animal data. In the present study 15 surfactants and eight fatty acid substances were tested on human skin in the assay. Three surfactants and four fatty acids were classified as corrosive in vivo. In the assay, the three surfactants were identified as corrosive, but all the fatty acid substances were found to be non-corrosive. It is suggested that the fatty acid substances labeled as corrosive on the basis of animal data are over-labeled, i.e., in humans the substances would not lead to a corrosive effect. It is envisaged that the in vitro human skin corrosivity test will be used in conjunction with human patch tests, currently being developed. This approach should allow a redn. in animal testing and a more relevant classification of skin irritation potential to humans.

46
Preat V, Thysman S. TRANSDERMAL IONTOPHORETIC DELIVERY OF SUFENTANIL. Int J Pharm 1993;96(1-3):189-96.

Iontophoresis promotes the penetration of charged and uncharged mols. through the skin using an elec. current application. In vitro assays were performed to investigate the influence of several elec. and physicochem. parameters on the transdermal permeation of sufentanil. Continuous current application strongly enhanced sufentanil flux through hairless rat skin as compared to passive diffusion. D.c. was more potent than pulse current to promote sufentanil transdermal permeation. An enhancement in c.d. applied induced an increase in the flux of the drug. When current application was terminated before the end of the experiment, the flux decreased but remained higher than diffusion flux. The pH of the medium affected diffusion and iontophoretic fluxes: in contrast with diffusion, acidic pH was more efficient for iontophoresis. An enhancement of drug concn. enhanced the iontophoretic flux. Application of d.c. or pulse current induced similar changes in skin permeability to water.

47
Lambert WJ, Kudla RJ, Holland JM, Curry JT. A BIODEGRADABLE TRANSDERMAL PENETRATION ENHANCER BASED ON N-(2-HYDROXYETHYL)-2- PYRROLIDONE. I. SYNTHESIS AND CHARACTERIZATION. Int J Pharm 1993;95(1-3):181-92.

Penetration enhancers represent a popular method of increasing drug flux through the skin for local or systemic activity. Unfortunately, it is thought that the local irritation commonly seen with penetration enhancers is directly related to the penetration enhancement ability of the enhancer. A potential method of avoiding irritation while maintaining enhancement is to utilize a 'soft' enhancer which is metabolized to inert components in the viable epidermis after achieving the desired effect in the dead cells of the stratum corneum. In the present report, model fatty acid esters of N-(2-hydroxyethyl)-2- pyrrolidone were synthesized in order to test this approach. A 2-order of magnitude increase in permeability for hydrocortisone through mouse skin was achieved in vitro with these enhancers. The ester linkage was readily cleaved by hydrolytic enzymes in plasma and skin homogenates, while having relatively good soln. stability at neutral and slightly acidic pH. Finally, these agents appear to have much less irritation

potential than traditional penetration enhancers. Thus, this novel class of enhancers has a high potential for increasing drug flux without irritation in transdermal drug delivery.

48
Ren D, Li H. THE EFFECTS OF ALCOHOL AND DETERGENT ON THE SKIN BARRIER FUNCTION. Zhonghua Laodong Weisheng Zhiyebing Zazhi 1993;11(3):156-8.

Skin barrier function was damaged by 70% EtOH as the disinfectant, but not by 0.01-1% detergent in rat skin immersion test in vitro.

49
Rigano L, Giogilli S. EVALUATION OF THE EFFECTS OF SOLID DETERGENTS ON SKIN: A SENSORIAL AND INSTRUMENTAL APPROACH. Comun Jorn Com Esp Deterg 1993;24:547-65.

Many instrumental methods can be applied to the measure of the irritative power and skin barrier function modifications that take place during detergent exposure. In order to have a better comprehension of skin equil. changes and product cleaning performances, different contemporary measures should be carried out. Many variables influence the results and their interpretation; skin color, active materials content and compn., presence of anti-irritant mols., phys. form of the detergents, type of analyzed cutaneous property. The results obtained in the evaluation of many cosmetic detergent mixtures and formulations are described, together with some correlations with an in-vitro toxicol. method and physical measures. The main problems in defining a protocol for sensorial evaluations of solid detergents for skin are also described.

50
Tiemessen H. PERCUTANEOUS ABSORPTION - ANIMAL SKIN MODELS VERSUS IN VITRO MODELS USING HUMAN SKIN. Paperback APV 1993;31,Dermal and Transdermal Drug Delivery:101-17. (REFS 25)

The skin is recognized more and more as an entrance route for xenobiotics into the human body. Transdermal drug delivery systems (TDS) continue to be developed from drugs acting systemically and as such constitute a major dosage form. Enhanced dermal drug delivery is

also of high interest at the moment with new dermal delivery systems like niosomes and liposomes. To evaluate the risk of toxic compounds to which the human skin can be exposed it is very important to know the extent of dermal and transdermal penetration. In research and development in aforementioned fields, it is of vital important to have access to exptl. models in the lab. which allow for a prediction of the dermal- and transdermal transport in man after exposure to xenobiotic. This paper intends to discuss methods and models used for the fields mentioned. Special attention will be given to the newly developed human stratum corneum/silicone membrane methods which are presented as powerful and very practical tools in both research and development.

51
Reece BT, Deeds D, Rozen M. AN IN VITRO METHOD FOR SCREENING SUNSCREEN FORMULATIONS FOR SUN PROTECTION FACTOR USING A FULL-THICKNESS SKIN MODEL. J Soc Cosmet Chem 1992;43(6):307-12.

No abstract.

52
Kemppainen BW, Terse P, Madhyastha MS, Lenz SD, Palmer WG, Reifenrath WG. IN VITRO ASSESSMENT OF IN VIVO DAMAGE TO THE BARRIER PROPERTIES OF PIG SKIN CAUSED BY A COMPLEX MIXTURE. J Toxicol, Cutaneous Ocul Toxicol 1993;12(3):239-48.

The purpose of this study was to determine if an in vitro method can be used to measure changes in the barrier properties of skin caused by in vivo or in vitro topical exposure to a test chemical. The effect of a test chemical (liq. gun propellant [LP], a highly irritating mixt. of hydroxylammonium nitrate, triethanolammonium nitrate, and water) on the barrier function of skin was assessed in vitro by measuring the penetration of [14C]benzoic acid following exposure to LP. Weanling pigs were topically exposed to single doses (25 muL/cm2) of saline (control) or LP. LP-induced changes in the barrier properties of the skin were detd. by measuring the permeability to [14C]benzoic acid. After 1-5 days, pigs were euthanized and skin sections excised from the sites of application. Skin sections were mounted in in vitro penetration chambers to measure cumulative 24 h penetration of [14C]benzoic acid. Topical treatment

with undiluted LP resulted in an 8.2-fold increase in permeability to [14C]benzoic acid at 1 day after exposure. The permeability declined progressively during the succeeding 4 days. In a parallel study, untreated skin was excised, placed in penetration chambers, and then exposed to saline or LP for 1 day. In vitro skin exposure to LP resulted in a 3.9-fold increase in penetration of [14C]benzoic acid. These studies demonstrate that the effect of LP on skin barrier properties is greatest at 1 day postexposure and steadily decreases thereafter and that both in vivo and in vitro dermal exposure to LP alters the barrier properties of skin. This in vitro method, which is the quantitation of transdermal transport of benzoic acid, can be used to assess the effect of chemical or physical agents on barrier function and time course of return to normal barrier function of skin.

53
Muller-Decker K, Furstenberger G, Marks F. DEVELOPMENT OF AN IN VITRO ALTERNATIVE ASSAY TO THE DRAIZE SKIN IRRITANCY TEST USING HUMAN KERATINOCYTE-DERIVED PROINFLAMMATORY KEY MEDIATORS AND CELL VIABILITY AS TEST PARAMETERS. In Vitro Toxic: A Journal of Molecular and Cellular Toxicology 1992;5(4):191-209. (REFS 68)

An in-vitro alternative assay to the animal Draize skin irritancy test was described. The assay used human foreskin derived keratinocyte cell line HPKII. Three mechanistically independent in-vitro test parameters of irritancy which reflected major characteristics of acute inflammation were proposed. These included the concentration of key mediators of the eicosanoid cascade, the concentration of interleukin-1-alpha released upon the in-vitro challenge of cells with test substances, and the toxic insult on these cells. In initial tests, conditions were optimized with respect to serum concentration and cell density. The cells were found to release arachidonic-acid, prostaglandin- E2, and the proinflammatory cytokine interleukin-1- alpha upon challenge with selected exogenous irritant compounds. The authors conclude that the measurement of proinflammatory mediator release and cytotoxicity in these cells may prove to be suitable as a test system for the prediction of the irritant potential of chemicals. Findings on structurally unrelated pharmacologically relevant skin irritants are being compared to those of related non or mildly irritating substances.

54
Song J, Liu X, Shi Y, Zhou L, Hanying Y. THE FORMULA DESIGN AND THE SCREEN MODEL IN VITRO FOR PRAZIQUANTEL SPRAY. Zhongguo Yaoke Daxue Xuebao 1993;24(2):109-11.

A drug dissolution instrument and TLC were used as the main methods for establishing a simplified model. The latter was employed in search of water-resisting skin-care formula. Eighteen formulas of praziquantel spray, which may be divided into four types, were screened. The best water-resisting formula was one contg. a proper penetration enhancer. A good protective effect was shown in animal prophylactic tests.

55
Sanghvi PP, Collins CC. COMPARISON OF DIFFUSION STUDIES OF HYDROCORTISONE BETWEEN THE FRANZ CELL AND THE ENHANCER CELL. Drug Dev Ind Pharm 1993;19(13):1573-85.

The Franz diffusion cell remains a popular method to study diffusion of transdermal drug delivery systems through membranes. Recently, the Enhancer cell, a new device for in vitro transdermal drug diffusion testing, has been developed. The purpose of this study was to evaluate the Enhancer cell for in vitro transdermal diffusion of hydrocortisone from an ointment using a synthetic membrane and a biol. membrane and compare it to the traditionally employed Franz cell. The Enhancer cell utilizes existing USP dissolution equipment (USP App. II). Results show a higher cumulative release from the enhancer cell as compared to the Franz cell. The Enhancer cell demonstrated more durability and was easier to use during experimentation and after completion of the expt. no apparent change was observed in the condition of the ointment or the skin when compared to the Franz cell.

56
Kadir R, Tiemessen HL G, Ponec M, Junginger HE, Bodde HE. OLEYL SURFACTANTS AS SKIN PENETRATION ENHANCERS. EFFECTS ON HUMAN STRATUM CORNEUM PERMEABILITY AND IN VITRO TOXICITY TO CULTURED HUMAN SKIN CELLS. Drugs Pharm Sci 1993;59(Pharmaceutical Skin Penetration Enhancement):215-27.

Oleic acid and oleyl alcohol are very effective

penetration enhancers for the delivery of nitroglycerin through human stratum corneum. It was shown that, compared with EO-10-oleyl ether, EO-2 and EO-5-oleyl ether solns. in propylene glycol significantly increase nitroglycerin transport through isolated human stratum corneum. In addition, EO-5-oleyl ether shows a greater enhancing effect than its ester analogs. In the in vitro cell toxicity assay the EO-5- and EO-10 oleyl esters appear to be less toxic than their ether analogs, probably due to enzymic degrdation by the cultured skin cells. This observation immediately prompts the idea of developing biodegradable penetration enhancers. The results of this study clearly show that highly effective penetration enhancers are not necessarily more toxic than less effective ones. In other words: there is no compelling reason for assuming a general correlation between enhancement properties on the one hand, and skin irritancy (primary cell toxicity) on the other.

57
Ping Q, Sun G, Chen L, Zhao P, Pan Y. EFFECT OF PENETRATION ENHANCERS ON IN VITRO PERMEABILITY OF LEVONORGESTREL IN POSTCOITAL TRANSDERMAL CONTRACEPTIVE PATCH THROUGH EXCISED MOUSE SKIN. Zhongguo Yiyao Gongye Zazhi 1993;24(2):58-61.

The effect of penetration enhancers on the in vitro permeability of levonorgestrel (I) through excised mouse skin under various conditions was evaluated using HPLC-UV. When a satd. soln. of I in ethanol was used as donor phase and the skin was pretreated with enhancers for 4 h, propylene glycol(PG) and PVP-oleic acid showed significant enhancing ability, while oleic acid, laurocapram, phthalate had the negative action. When unpretreated skin was employed, the enhancers, such as PG, dissolved in satd. water soln. of I showed almost no promoting effect. EVA-matrix patches fabricated by solvent casting method contg. I and enhancer adhered to the mouse skin, the enhancing effect of all enhancers except oleic acid was in good agreement with the results of pretreated skin.

58
Beck H, Bracher M, Faller C, Hofer H. COMPARISON OF IN VITRO AND IN VIVO SKIN PERMEATION OF HAIR DYES. Cosmet Toiletries 1993; 108(6):76-83.

The cutaneous permeation of 8 oxidative and 10

semipermanent hair dye components was determined in vivo (rat) and in vitro (pig skin) in order to investigate the in-vivo/in-vitro correlation of the 2 test systems. An excellent correlation was found after approx. application of the semipermanent nitro dyes in a hair-dyeing product base. The oxidative (permanent) hair dyes were applied in 3 different vehicles with different soly. properties: Appropriated hair-dyeing mixts. with hydrogen peroxide and reactive compns. for oxidative coupling reactions. The same mixture without hydrogen peroxide, and for comparative reasons, water alone. The in-vivo/in-vitro correlation of cutaneous permeation for these was still satisfactory, but somewhat lower than with semipermanent dyeing compns. The overall correlation coeff. of semipermanent and oxidative hair dyes (24 applications total) was R = 0.94. In every case, the in-vitro pig skin permeation was lower than the in-vivo permeation in rats. It is concluded that the described method of in-vitro cutaneous permeation with pig skin is a good model for the prediction of in-vivo skin permeation of oxidative and semipermanent hair dyes.

59
Yang JJ, Krueger AJ. EVALUATION OF TWO COMMERCIAL HUMAN SKIN CULTURES FOR IN VITRO PERCUTANEOUS ABSORPTION. In Vitro Toxicology. A Journal of Molecular and Cellular Toxicology 1992;5(4):211-217. (REFS 10)

The in-vitro skin permeation properties of two commercial human skin cultures were evaluated in comparison to those of human abdominal skin. The human skin models that were studied included TESTSKIN(TM) Living Skin Equivalent and Skin2(TM) Barrier Function Model ZK2000. The four substances selected for testing were water, toluene (108883), carbazole (86748) and benzo(a)pyrene (50328). Percutaneous absorption was determined by measuring the accumulation of radioactivity in the designated receptor fluid over time. While similar values were obtained for the percutaneous absorption of the four substances by the skin models, these preparations proved to be generally more permeable than normal skin. The greatest effect, a ten to 14 fold elevation in flux rate, was observed with water. Increased water permeation was observed when 6% Volpo-20 was used as the receptor fluid in place of culture medium. Applications of the cultured human skin model were considered. The authors conclude that the commercial preparations appear to be suitable

for the in-vitro determination of the relative absorption properties of chemical compounds under various conditions; relative permeability may vary considerably from one compound to another.

60
Garmyn M, Yaar M, Boileau N, Backendorf C, Gilchrest BA. EFFECT OF AGING AND HABITUAL SUN EXPOSURE ON THE GENETIC RESPONSE OF CULTURED HUMAN KERATINOCYTES TO SOLAR-SIMULATED IRRADIATION. J Invest Derma 1992;99(6):743-748. (REFS 46)

The effects of aging and habitual sun exposure (photoaging) on the genetic response of human keratinocytes to simulated solar radiation were studied in-vitro. Keratinocytes harvested from newborn foreskin, breast skin from 19 to 48 year old adults (young adults), and sun exposed or unexposed skin from the upper arms of 63 to 73 old donors (old adults) were exposed or sham exposed to 16 millijoules per square centimeter (cm2) ultraviolet light from a 1 kilowatt xenon arc filtered to produce an ultraviolet irradiance of 2x10(-4) watt/cm2. This exposure was chosen to simulate an exposure that would cause a moderate sunburn. The cells were examined for a change in morphology and number at various times up to 72 hours post irradiation. Intracellular mRNA was extracted 0, 4, 8, 24, and 48 hours post exposure. The mRNA was analyzed by Northern blotting using cDNA probes that encoded for the protooncogenes c-fos and c-myc, the GADD153 gene which was a marker for growth arrest and DNA damage, interleukin-1a, interleukin-1b, interleukin-1ra (IL1ra), and the SPR2 gene which was a marker for ultraviolet-C induced lethal damage. Ultraviolet radiation did not significantly affect cell morphology. Sham exposed keratinocytes from newborn and young adult skin showed a four fold increase in cell number over the 72 hour post irradiation period. Keratinocyte counts did not change significantly in irradiated skin. Baseline expression of the SPR2 gene and IL1ra increased with increasing age. Irradiation decreased baseline expression of the SPR2 gene and IL1ra and decreased inductance of the c-fos protooncogene in photoaged keratinocytes from old adults relative to keratinocytes from unexposed skin. None of the other genes or cytokines were significantly affected by ultraviolet irradiation. The authors conclude that photoaging may reflect, at least in part, a dedifferentiation of keratinocytes. When compounded by other ultraviolet induced DNA changes,

dedifferentiation may predispose photoaged skin to photocarcinogenesis.

61
Beck H, Bracher M, Faller C, Hofer H. COMPARISON OF IN VITRO AND IN VIVO SKIN PERMEATION OF HAIR DYES. Cosmet Toilet 1993; 108(6):76-83. (REFS 13)

To investigate the correlation of in vivo (rat) and in vitro (pig skin) testing of skin permeation of hair dye components, 8 oxidative and 10 semipermanent hair dye components were tested using both techniques. Results indicated that the in vitro permeation method was found to be a good model for the prediction of skin permeation of oxidative and semipermanent hair dyes.

62
Wester RC, Maibach HI, Sedik L, Melendres J, Wade M. IN VIVO AND IN VITRO PERCUTANEOUS ABSORPTION AND SKIN DECONTAMINATION OF ARSENIC FROM WATER AND SOIL. Fundam Appl Toxic 1993; 20(3):336-340. (REFS 14)

Results of a study to determine the percutaneous absorption of arsenic (7440382) as arsenic-acid (7778394) from water and soil into and through human skin using in-vitro and in-vivo technology were presented. Female rhesus-monkeys were given an intravenous arsenic-73 dose and the majority was excreted the first day with 80% being recovered in the urine over a 7 day period of time. With topical administration for 24 hours, absorption of the low dose from water was 6.4% and 2.0% from the high dose. In-vitro percutaneous absorption of the low dose from water with human skin resulted in 24 hour receptor fluid accumulation of 0.93% dose and skin concentration of 0.98%. The total in-vitro value was 1.9%, less than the in-vivo value of 6.4% in the Rhesus-monkey. From soil, the in-vitro accumulation was 0.8% and the in-vivo was 4.5% in the Rhesus-monkey. Washing with

soap and water readily removed residual skin surface arsenic, both in-vivo and in-vitro. The binding of arsenic to powdered human stratum corneum and soil were similar, which may indicate why arsenic absorption was similar from both water and soil. The authors suggest that the powdered human stratum corneum partition coefficient model may provide an easy method for such predictions.

63
Rhoads LS, Cook JR, Patrone LM, Van Buskirk RG. A HUMAN EPIDERMAL MODEL CAN BE ASSAYED EMPLOYING A MULTIPLE FLUORESCENT ENDPOINT ASSAY AND THE CYTOFLUOR 2300. J Toxicol Cutaneous Ocul Toxicol 1993;12(2):87-108.

A human epidermal model (HEM) was developed that could

be rapidly and automatically assayed in the CytoFluor 2300 (Millipore Corporation, Bedford, MA) spectrofluorometer using multiple site-and activity-specificity fluorescence probes. The HEM was cultured on the optically translucent Millipore Millicell CM microporous membrane. Application of a variety of fluorescent dyes to this membrane without the HEM revealed negligible nonspecific dye association. The HEM was differentiated on a cross-linked collagen matrix and the latter was also found to retain less dye than the HEM. Feasibility experiments using the site/activity-specific dyes calcein-AM (plasma membrane integrity indicator), sodium fluorescein (epidermal permeability indicator), 5-chloromethylfluorescein diacetate-acetoxymethyl ester (CMFDA-AM; intracellular glutathione level indicator), rhodamine 123 (mitochondrial activity indicator), neutral red (lysosomal integrity indicator), Fluo3-AM (intracellular calcium indicator), and a similar human epidermal model, the Organogenesis indicator), and a similar human epidermal model, the Organogenesis Testskin living skin equivalent (LSE), indicated that in vitro human epidermis might be amenable to automated analysis in the CytoFluor 2300. To determine if these fluorescent probes might reveal mechanisms underlying cytotoxicity, normal human epidermal keratinocyte (NHEK) monolayers were exposed to the single arm mustard, 2-chloroethylethylsulfide (CEES) and labeled with the aforementioned fluorescent probes. The data reveal that there is a dose- and time-dependent alteration in cellular activities due to mustard toxicity. The fact that these HEMs can be analyzed automatically using the CytoFluor 2300 and that changes in specific physiological parameters can be assessed using multiple fluorescent dyes suggests that this process might be a high throughput manner in which to screen further skin irritants.

64
Burnham K, Pickard S, Hudson J, Voss T. REQUIREMENTS

FOR LANGERHANS' CELL DEPLETION FOLLOWING IN VITRO EXPOSURE OF MURINE SKIN TO ULTRAVIOLET-B. Immunology 1993; 79(4):627-632.

Langerhans' cells found within the skin and mucous membranes are critical regulators of antimicrobial and allergic responses. Therefore, the depletion of these cells following exposure of skin to solar ultraviolet radiation (UV) has direct functional consequences on immunity within this tissue. In order to understand how Langerhans' cell depletion is regulated following exposure of skin to medium-wave UV (UVB), the role of second messengers in these responses was investigated using a novel in vitro system. This was accomplished by analysing the expression of a specific marker associated with Langerhans' cells (ATPase) among the epidermal portion of cultured sections of mouse skin following treatment with inhibitors specific for second messenger components and subsequent exposure to UVB. In this study, inhibitors of guanosine triphosphate (GTP) binding proteins, H-8, pertussis toxin and cholera toxin as well as inhibitors of RNA and protein synthesis were all capable of blocking Langerhans' cell depletion in response to UVB treatment. In contrast, an inhibitor of protein kinase C (H-7) was incapable of

specifically blocking depletion following treatment with this physical agent. These results suggest that Langerhans' cell depletion mediated by UVB may involve a pertussis and cholera toxin-sensitive G protein as well as de novo protein synthesis.

65
Moody RP. In vitro dermal absorption of pesticides: A cross-species comparison including testskin. J Toxicol Cutaneous Ocul Toxicol 1993;12(2):197-202.

Dermal absorption studies with living animals have several disadvantages. The present study reports the use of our 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 compounds being chosen for their wide range of lipophilicities. Absorption (percentage recovery in receiver solution) in the human-derived tissue-cultured skin. Testskin, was similar to that in pig skin for three of the four test compounds. Testskin 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 Testskin. Total cumulative absorption of CYA by 24 hr in Testskin and human skin was 0.02 mug CYA/cm2 in both cases.

66
Nelson D, Gay RJ. EFFECTS OF UV IRRADIATION ON A LIVING SKIN EQUIVALENT. Photochem Photobiol 1993;57(5):830-837.

The Living Skin Equivalent (LSE) is an organotypic coculture composed of human dermal fibroblasts interspersed in a collagen-containing matrix and overlaid with human keratinocytes forming a stratified epidermis. The LSE has a dry, air-exposed epidermal surface suitable for the application of oils, creams and emulsions. These features suggested its feasibility as an in vitro skin model for studying the protective effects of sunscreens. Using the thiazolyl blue (MTT) conversion assay as a measure of mitochondrial function, the extent of cytotoxicity induced by various doses of UV-R (280-400 nm) or UV-A (320-400 nm) was evaluated in the LSE. The doses of UV radiation the caused 50% reductions in MTT conversion (UV-R50 or UV-A50) in different lots of LSE were 0.053:0.021 J/cm2 (n=29) and 11.6:4.9 J/cm2 (n=17) for UV-R and UV-A, respectively. The protective effects of an 8% homosylate standard and of five UV-A sunscreens, topically applied to the LSE, were determined and compared with their reported protection factors in human skin. Morphological changes and the release of proinflammatory mediators (interleukin-1-alpha, tumor necrosis factor-alpha and prostaglandin E2) implicated in UV-induced erythema were also demonstrated in the

LSE exposed to UV-A or UV-B. The data suggest that the LSE can be used for studying the effects of UV radiation on skin and may have utility for assessing the efficacy of certain sunscreens against UV-B and UV-A.

67
Hughes MF, Shrivastava SP, Fisher HL, Hall LL. COMPARATIVE IN VITRO PERCUTANEOUS ABSORPTION OF P-SUBSTITUTED PHENOLS, THROUGH RAT SKIN USING STATIC AND FLOW-THROUGH DIFFUSION SYSTEMS. Toxicol in vitro 1993;7(3):221-227.

The objective of this study was to compare the in vitro percutaneous absorption of phenol and eight p-substituted phenolic derivatives using the static and flow-through diffusion systems. The variability of the cumulative percentage absorption data was also examined. Clipped dorsal skin was removed from female Fischer 344 rats (90 days old), cut on a dermatome to approximately 350 mum thick, placed in a diffusion cell and treated with chemical (4 mug/cm2, ethanol). The 72-h cumulative absorption into the receptor fluid for the phenols in both systems ranged from 15.4 to 97.6% of the dose. Absorption was greater than 70% in both systems for phenols with a log octanol:water partition coefficient (log P) value between 1.4 and 3.5. The percentage of the dose remaining in the skin and washed from the skin and cell top at 72 hr in both systems ranged from 1.8 to 56.2% and from 0.7 to 45.2%, respectively. There were significant differences in absorption between the two systems with five of the phenols. Absorption was significantly lower for acetamido-, chloro- and cyanophenol in the static system and phenol and heptyloxyphenol in the flow-through system. However, the differences in absorption for phenol and chlorophenol were small (< 4%) and may not be that biologically relevant. Phenols absorbed the least (< 70%), were also on the extremes of the log P range examined and had the most variable absorption data. Both systems appear to be equivalent means to investigate in vitro percutaneous absorption of chemicals, but it is critical to recognize that the data can vary, especially for chemicals not absorbed well.

68
Kemppainen BW, Terse P, Madhyastha MS, Lenz SD, Palmer WG, Riefegrath WG. IN VITRO ASSESSMENT OF IN VIVO DAMAGE TO THE BARRIER PROPERTIES OF PIG SKIN CAUSED BY A COMPLEX MIXTURE. J Toxicol Cutaneous Ocul Toxicol 1993;12(3):239-248.

The purpose of this study was to determine if an in vitro method can be used to measure changes in the barrier properties of skin caused by in vivo or in vitro topical exposure to a test chemical. The effect of a test chemical (liquid gun propellant (LP), a highly irritating mixture of hydroxylammonium nitrate, triethanolammonium nitrate, and water) on the barrier function of skin was assessed in vitro by measuring the penetration of (14C)benzoic acid following exposure to LP. Weanling pigs were topically exposed to single

doses (25 mul/cm2) of saline (control) or LP. LP-induced changes in the barrier properties of the skin were determined by measuring the permeability to (14C)benzoic acid. After 1-5 days, pigs were euthanized and skin sections excised from the sites of application. Skin sections were mounted in in vitro penetration chambers to measure cumulative 24 hr penetration of (14C)benzoic acid. Topical treatment with undiluted LP resulted in an 8.2-fold increase in permeability to (14C)benzoic acid at 1 day after exposure. The permeability declined progressively during the succeeding 4 days. In a parallel study, untreated skin was excised, placed in penetration chambers, and then exposed to saline or LP for 1 day. In vitro skin exposure to LP resulted in a 3.9-fold increase in penetration of (14C)benzoic acid. These studies demonstrate that the effect of LP on skin barrier properties is greatest at 1 day postexposure and steadily decreases thereafter and that both in vivo and in vitro dermal exposure to LP alters the barrier properties of skin. This in vitro method, which is the quantitation of transdermal transport of benzoic acid, can be used to assess the effect of chemical or physical agents on barrier function and time course of return to normal barrier function of skin.

69
Nair X, Parab P, Suhr L, Tramposch KM. COMBINATION OF 4-HYDROXYANISOLE AND ALL-TRANS RETINOIC ACID PRODUCES SYNERGISTIC SKIN DEPIGMENTATION IN SWINE. J Invest Dermatol 1993;101(2):145-9.

A combination of 4-hydroxyanisole (4HA) and all-trans retinoic acid (TRA) was found to synergistically cause moderate to complete depigmentation of Yucatan swine skin. Two hyperpigmentation models were used: Natural dark-skinned swine, a potential model for melasma-like disorders, and ultraviolet light-stimulated hyperpigmentation, a model of solar lentigines. Test materials were applied twice daily, 5 d/week, to dorsal flank skin. Application sites were graded at weekly intervals for skin color using a 0 to 4 grading scale. After 8 weeks of treatment of naturally dark swine skin, a combination of 2% 4HA and 0.01% TRA produced grade 2 hypopigmentation (definite but moderate hypopigmentation). In contrast, 2% 4HA alone or 0.01% TRA alone did not produce significant hypopigmentation. After cessation of treatment, the 4HA/TRA-treated sites reverted to normal color within 7-12 weeks. The 4HA/TRA combination completely reversed the

hyperpigmentation induced by ultraviolet light after 8 weeks of treatment. In vitro skin-penetration studies using hairless mouse and human skin show that skin penetration of 4HA was not significantly affected by adding 0.01% TRA. These data suggest that the observed synergy is not due to enhanced bioavailability of 4HA. We have demonstrated that combining low concentrations of 4HA and TRA results in effective skin lightening without causing irreversible depigmentation and with minimal local skin irritation.

DEVELOPMENTAL TOXICITY

70

Fort DJ, Rayburn JR, Bantle JA. EVALUATION OF ACETAMINOPHEN- INDUCED DEVELOPMENTAL TOXICITY USING FETAX. Drug Chem Toxicol 1992;15(4):329-50.

Potential mechanisms of acetaminophen-induced developmental toxicity were evaluated using FETAX (Frog Embryo Teratogenesis Assay-Xenopus). Early Xenopus laevis embryos were exposed to acetaminophen for 96-h in two definitive concentrations-response assays with and without an exogenous metabolic activation system (MAS). Two static renewal tests of acetaminophen and the MAS treated with carbon monoxide, cimetidine, ellipticine, diethyl maleate, and supplemented with glutathione were also performed. Addition of the MAS decreased the 96-h LC50 and EC50 (malformation) values of unactivated acetaminophen 3.9-fold and 7.1-fold, respectively. Addition of the carbon monoxide- and ellipticine-inhibited MAS, as well as the glutathione-supplemented MAS decreased the developmental toxicity of activated acetaminophen to levels near that of the unactivated parent compound. Cimetidine-inhibited MAS also reduced the developmental toxicity of acetaminophen, but not to the extent observed with the carbon monoxide- and ellipticine-inhibited, or glutathione-supplemented MAS. Addition of the diethyl maleate-treated MAS substantially increased the developmental toxicity of acetaminophen. Results indicate that a highly reactive intermediate formed as the result of MFO-mediated metabolism (possibly P-448) significantly increased the developmental toxicity of acetaminophen. Glutathione was also found to play a major role in intermediate detoxification in vitro.

71

Brown NA. HAZARD IDENTIFICATION IN DEVELOPMENTAL TOXICOLOGY - THE ROLE OF NONWHOLE MAMMAL TESTS. Reprod

Toxicol 1992;6(2):178.

Evaluating the toxic risks of chemical and physical agents is clearly a multistep process. Many ways of dividing the process have been proposed. The US National Research Council (1983) suggestion of hazard identification, dose-response assessment, exposure assessment, and risk characterization is widely used. Risk communication, management, and perception are subsequent problems of a quite different nature. For the present purposes, I would like to consider three steps: 1) recognition that an agent may pose a risk-hazard identification; 2) collection and analysis of all information available on the toxic effect, including dose-response and pharmacokinetics-hazard characterization; 3) estimation of the probability that the effect would be induced in humans under particular exposure conditions-risk characterization. Most recognized human developmental toxicants were detected retrospectively from clinical or epidemiologic observation. Our aim should be to ensure that this becomes rare or nonexistent. A major problem is to identify especial developmental hazards from among the thousands of extant untested agents, new mixtures, and novel chemicals. Most agencies currently will not use results from nonmammalian and in vitro tests in hazard identification. I will argue that alternatives to orthodox whole mammal testing should have an important role in this process, in particular in "primary screening," although much work has still to be done to refine these methods. It appears that the past 10 years have produced relatively few advances in this use of alternative tests. I will suggest that this is because too much effort has been concentrated on so-called "validation" studies. I will propose different strategies, both for the immediate application of existing techniques and for method development. Further, I will also argue that some in vitro models can contribute valuable information to hazard and risk characterization, even, ironically, to pharmacokinetic aspects. The possibility of precisely controlling exposure concentration and duration and eliminating metabolic complexity are very useful in some circumstances. I will use two specific examples of the use of in vitro data in this "secondary testing" mode.

72
Mitra A, Hilbelink DR, Dwornik JJ, Kulkarni A. A NOVEL MODEL TO ASSESS DEVELOPMENTAL TOXICITY OF DIHALOALKANES IN HUMANS: BIOACTIVATION OF

1,2-DIBROMOETHANE BY THE ISOZYMES OF HUMAN FETAL LIVER GLUTATHIONE S-TRANSFERASE. Teratogenesis Carcinog Mutagen 1992;12(3):113-27.

Glutathione S-transferase (GST) isozymes from human fetal liver (16-18 weeks gestation) were purified by affinity chromatography followed by ion-exchange high performance liquid chromatography (HPLC). The purified isozymes were used to investigate toxicity of 1,2-dibromoethane(EDB) in an in vitro model of rat embryos in culture as passive targets. At least five isozymes of GST were found in the human fetal liver. Two anionic forms [pI values 5.5 (P-2) and 4.5 (P-3)] and one basic form [pI value 8.7 (P-6)] were clearly separated. The presence of two near-neutral forms was also identified. All the isozymes of the human fetal liver GSTs tested metabolized EDB (specific activities were 2.1, 7.0, and 2.0 mumol of GSH consumed/min/mg protein for P-2, P-3, and P-6 isozymes, respectively). Covalent binding of EDB to DNA and protein was 144% and 212% higher, respectively, with the P-3 anionic isozyme when compared to the P-6 basic isozyme of GST. No covalent binding to either protein or DNA was observed with the P-2 isozyme. EDB bioactivation by the GST isozyme P-3 (15 units; 1 unit = 1 nmol of GSH consumed/min) resulted in toxicity to cultured rat embryos. Significant reductions of crown rump length, yolk sac diameter, and the composite score of morphological parameters (Brown and Fabro method) were observed. The central nervous system, optic and olfactory systems, and the hind limb were most significantly affected. The results of this investigation suggest that EDB may be classified as a suspected developmental toxicant in humans.

73
Klug S, Hinz N, Kastner U, Neubert D. TCDD INHIBITS CLOSURE OF THE SECONDARY PALATE IN MICE: DOES ALTERATION OF EXTRACELLULAR MATRIX COMPONENTS PLAY A ROLE? Teratology 1992;46(3):24A.

It is well known that TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) leads to the induction of cleft palate in mice. However, little is known about the mode of action. Since we have established a system which allows simulatation of closure of the secondary palate in vitro (Acta Anat 1990; 137: 59-64), we consequently studied the way of palate closure and the effects of TCDD in vitro. First results gave evidence that TCDD interferes with the

palate closure in the latest phase (after attachment of the shelves). For this reason we decided to study the morphological structures and their alteration during closure of the palate by using polyclonal antibodies produced at our institute. The following antibodies were used: against collagen type I, collagen type VII, fibronectin and against laminin. Staining of the cultured palate anlagen at varying stages of palate closure with the later antibodies led to the following: 1. Evidence that TCDD exposure induces an increased expression of collagen type I in the shelves. 2. In contrast to the control, not yet identified cellular material containing collagen type VII was seen after attachment of the shelves between the two basement membranes. 3. In contrast to the control, in the area of shelf fusion fibronectin was packed less densely. 4. Staining with laminin AB revealed in the treated palates a non-parallel and interrupted course of the two basement membranes, whereas in the control the two basement membranes were attached parallel and already desintegrated in some regions. These findings are yet to be confirmed on a larger scale.

74
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.

75
Tsuchiya T, Igarashi Y, Nakamura A. DEVELOPMENTAL TOXICITY OF URETHANE: IN VITRO TESTS USING VARIOUS EMBRYONIC CELLS AND LUNG ORGANS IN CULTURE. Teratology 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.

76
Smith EE, Duffus EA, Small MH. EFFECTS OF PATULIN ON POSTIMPLANTATION RAT EMBRYOS. Arch Environ Contam

Toxicol 1993;25(2):267-270.

Patulin (PAT), a highly toxic, carcinogenic, heterocyclic lactone is produced by a variety of fungal species, including Penicillium and Aspergillus. This compound has been isolated from various apple products and is stable in apple and grape juice and dry corn. It has been reported to be cytotoxic and to exert adverse influence on development in vivo in mice and merits further study and evaluation. In this study, whole rate embryo culture (WEC) was used to determine the teratogenic potential of PAT in vitro. Embryos were exposed to PAT-treated (0.00-62 muM) rat serum for 45 h. The embryos that were exposed to 62 muM PAT were not evaluated because they did not survive beyond 40 h of incubation. The results indicate that PAT induced a statistically significant reduction in protein and DNA content, yolk sac diameter, crown rump length, and somite number count. Patulin treatment also resulted in an increase in the frequency of defective embryos. Anomalies included growth retardation, hypoplasia of the mesencephalon and telencephalon, and hyperplasia and/or blisters of the mandibular process. Thus, the data from the present study provide further evidence supporting the conclusion that the whole rat embryo assay is a rapid and sensitive in vitro method than can be employed to pre-screen developmentally toxic mycotoxins.

EMBRYOTOXICITY
77
Goto Y, Noda Y, Mori T, Nakano M. INCREASED GENERATION OF REACTIVE OXYGEN SPECIES IN EMBRYOS CULTURED IN VITRO. Free Radical Biol Med 1993;15(1):69-75.

To obtain an actual proof of the increase in prodn. of reactive oxygen species within embryos, the authors have measured the level of H2O2 in individual embryos using a fluorimetric method. Mouse (ICR) pronuclear stage embryos from the oviducts were cultured for a specified time under various conditions in a medium to which 2',7'-dichlorodihydrofluorescin diacetate was added. After washing the embryos, the fluorescence emissions of the H2O2-dependent oxidative product in embryos were measured. The fluorescent emissions were lowest in embryos cultured under 5% O2 and highest under 40% O2(5% < 20% < 40%), just the inverse of the culture efficacy. The fluorescence emissions of embryos cultured in Ham's F-10, which contains hypoxanthine and transition metals such as Cu and Fe, were higher than

those cultured in BWW and alphaMEM, which do not contain these components (alphaMEM < BWW < Ham's F-10; again this is the inverse of the culture efficacy). The fluorescence emissions of embryos increased with the time of the exposure to visible light. L-Cysteine and thioredoxin, both of which have been shown to promote embryo development, decreased the fluorescence emissions of embryos. All of these results would provide direct evidence for the hypothesis that oxygen radicals are involved in the development blockage.

78
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 alkaline cell lysates. Ca2+ and Mg2+ enhanced, while Zn2+, the Ca2+ chelator ethylenglycol-bis(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, methyl 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 sensitisation to X-irradiation 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-chemical short-term tests in vitro.

79
Tempel K-H, 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):318-324.

Chicken embryo brain and liver cells in vitro exhibited spontaneous DNA fragmentation as determined by

viscometry of alkaline cell lysates. Ca2+ and Mg2+ enhanced, while Zn2+, the Ca2+ chelator ethylenglycolbis(beta-aminoethyl-ether)N,N,N'-etraacetic 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, methyl 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 sensitisation of X-irradiation 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-chemical short-term tests in vitro.

80
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 VPAand 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.

81
Canseco RS, Sparks AE, Pearson RE, Gwazdauskas FC. EMBRYO DENSITY AND MEDIUM VOLUME EFFECTS ON EARLY MURINE EMBRYO DEVELOPMENT. J Assist Reprod Genet 1992;9(5):454-7.

One-cell mouse embryos were used to determine the effects of drop size and number of embryos per drop for optimum development in vitro. METHODS: Embryos were collected from immature C57BL6 female mice superovulated with pregnant mare serum gonadotropin and human chorionic gonadotropin and mated by CD1 males. Groups of 1, 5, 10, or 20 embryos were cultured in 5-, 10-, 20-, or 40-microliters drops of CZB under silicon oil at 37.5 degrees C in a humidified atmosphere of 5% CO2 and 95% air. RESULTS: Development score for embryos cultured in 10 microliters was higher than that of embryos cultured in 20 or 40 microliters. Embryos cultured in groups of 5, 10, or 20 had higher development scores than embryos cultured singly. The highest development score was obtained by the combination of 5 embryos per 10-microliters drop. The percentage of live embryos in 20 or 40 microliters was lower than that of embryos cultured in 10 microliters. Additionally, the percentage of live embryos cultured singly was lower than that of embryos cultured in groups. CONCLUSIONS: Our results suggest that a stimulatory interaction occurs among embryos possibly exerted through the secretion of growth factors. This effect can be diluted if the embryos are cultured in large drops or singly.

82
Leach RE, Stachecki JJ, Armant DR. DEVELOPMENT OF IN VITRO FERTILIZED MOUSE EMBRYOS EXPOSED TO ETHANOL DURING THE PREIMPLANTATION PERIOD: ACCELERATED EMBRYOGENESIS AT SUBTOXIC LEVELS. Teratology 1993;47(1):57-64.

This study examined the effects of ethanol (EtOH) on mouse preimplantation development using an in vitro culture method. Embryos at the 1-cell, 2-cell, or 4-cell stage were exposed for 24 h to medium containing EtOH, then further cultured without EtOH to determine their ability to form blastocysts and to eventually hatch from the zona pellucida. EtOH exposure either arrested or enhanced normal development, depending on dose and embryonic stage of exposure. Exposure of 1-cell and 2-cell embryos to 1.6% (w/v) EtOH decreased blastocyst formation and hatching, and exposure of 1-cell embryos to 0.4% EtOH inhibited their development. At 0.1%, EtOH had an opposite effect, causing an increase in the percent blastocyst formation of treated 1-cell and 2-cell embryos. Neither inhibition nor stimulation of blastocyst formation occurred in 4-cell embryos exposed to 0.1-1.6% EtOH. Using an in vitro outgrowth model of implantation, embryos that reached the blastocyst stage were further tested for their ability to produce differentiated trophoblast cells. Blastocysts previously exposed to 0.1% EtOH during the 1-cell stage appeared to form adhesive trophoblasts earlier than control embryos, indicating that EtOH exposure can induce precocious differentiation of the trophoblast cells. The EtOH treated blastocysts contained significantly more cells than control blastocysts. These results indicate that EtOH can alter preimplantation development by either inhibiting or accelerating cell growth and differentiation.

83
Zucker RM, Elstein KH, Thomas DJ, Rogers JM. TOXICANT-SPECIFIC INDUCTION OF APOPTOSIS. Teratology 1993;47(5):398.

Apoptosis, or programmed cell death, plays an essential role in embryogenesis. Apoptotic cells characteristically form DNA "ladders" in electrophoretic gels as a consequence of the activation of endonucleases that preferentially degrade

nucleosomal linker sections of DNA. We used the rat thymus as an in vitro model to study the toxicant-specificity of apoptosis induction. Following mechanical dissociation, thymocytes from 3-6 week old rats were suspended in RPMI containing 10% fetal bovine serum, exposed to 10 uM methylprednisolone (MP) and/or 0.5-2.5 uM tributyltin (TBT), and incubated at 37 degrees C for 1-4 hr. Apoptosis induction was detected both by electrophoresis and by flow cytometric analysis of nuclear DNA content. We observed that while MP

gradually induced apoptosis in up to 40% of the cells by 4 hr, TBT caused a dose-dependent induction of apoptosis in almost all cells within 1 hr. Thereafter, TBT-exposed thymocytes exhibited increased resistance to detergent-mediated cytolysis, indicative of gradual membrane fixation. Furthermore, while pretreatment with the protein inhibitor cycloheximide completely inhibited induction by MP, induction by TBT was unaffected. However, following simultaneous exposure, MP delayed onset of apoptotic induction by TBT, and at low concentrations, TBT delayed onset of induction by MP. These data suggest that there exist at least two mechanisms of toxicant-induced apoptosis that act within different time frames. The potential for applying these findings and methods to the study of apoptosis in embryos will be discussed.

84
Nasr-Esfahani MH, Winston NJ, Johnson MH. EFFECTS OF GLUCOSE, GLUTAMINE, ETHYLENEDIAMINETETRAACETIC ACID AND OXYGEN TENSION ON THE CONCENTRATION OF REACTIVE OXYGEN SPECIES AND ON DEVELOPMENT OF THE MOUSE PREIMPLANTATION EMBRYO IN VITRO. J Reprod Fertil 1992;96(1):219-31.

Analysis over the first 48 h of development in vitro from the one-cell stage to the early four-cell stage indicated that (i) ethylenediaminetetraacetic acid (EDTA) exerts the major beneficial effect on culture to the blastocyst stage of F1 and MF1 embryos, (ii) glutamine assists development of MF1, but not F1, embryos to the blastocyst stage and probably functions as part of a metabolic response to oxidative damage to mitochondria and (iii) exposure to glucose at some time during early cleavage is essential for full development to blastocysts. None of the culture conditions examined affected significantly the increase in concentration of reactive oxygen species in late two-cell embryos in vitro, although F1 embryos in vitro often had lower peroxide concentrations than MF1 embryos. A decline in

oxygen tension from 20 to 50% had no consistent effect on culture to the blastocyst stage or production of reactive oxygen species. Aminooxyacetate, an inhibitor of transaminase activity, prevented non-blocking embryos from developing beyond G2 of the second cell cycle. It is concluded that the chelation of transitional metals provides the most effective method of overcoming the block to development in vitro.

85
Tsuchiya T, Eto K, Burgin H, Kistler A. MICROMASS CULTURE OF MIDBRAIN CELLS AND ITS RELEVANCE TO IN VITRO MECHANISTIC STUDIES. Senten Ijo 1992;32:105-16.

The relationship between ethylenethiourea (ETU)-induced malformations of cultured rat whole embryos and the alterations of midbrain (MB) cells was investigated and species-specific ETU-induced alterations between rat and mouse MB cells were determined. Herefore, we developed new methods for monitoring teratogenic activities in serum fluids without heat treatment. The serum samples were prepared from rats and mice given ETU, and ETU-teratogenicity was evaluated in both species. We determined that the different sensitivity of the midbrain of the rat and mouse may be the main reason that ETU was teratogenic in rats but not in mice. Next, we showed that MB-cultures are unsuitable for estimating the teratogenic potential of arotinoids. MB differentiation was adversely affected only at concentrations which caused cell death. Finally, we demonstrated that the embryolethal action of new herbicides is not detectable in the micromass teratogenic test. However, the V79 colony assay may be useful for preliminary screening of embryolethal effects of herbicides.

86
Piersma AH, Haakmat SA, Hagenaars AM. IN VITRO ASSAYS FOR THE IDENTIFICATION OF COMPOUNDS INTERFERING WITH EMBRYONIC CELL DIFFERENTIATION USING EMBRYONAL CARCINOMA CELL LINES. Teratology 1992;46(3):18A.

Embryonal carcinoma (EC) cell lines provide a continuous, stable and animal-free source of differentiated embryonic cells that are useful in studies of embryonic cell differentiation, and of the effects thereupon of chemicals. Two assays have been developed in which the interference of compounds with embryonic cell differentiation can be assessed.

Differentiation of visceral endoderm- derived epithelial cells from the F9 EC cell line (in aggregate culture in the presence of retinoic acid) was monitored via the production of alpha-fetoprotein, which was assayed in a quantitative ELISA. Formation of mesoderm-derived beating muscle cells from the P19 EC cell line (in coculture with END-2 endodermal cells) was monitored by microscopic observation. As an initial validation five pairs of compounds were tested in a double-blind protocol in both assays. Each pair consisted of pharmacologically or chemically similar compounds with diverse teratogenic properties. Compounds within any pair had different effects in the assays, indicating the discriminatory potential of these assays. Most discrepancies with in vivo teratogenicity could be explained in terms of solubility, kinetics, and end points. The results support the possible usefulness of the assays as prescreens in testing for interference with embryonic cell differentiation.

87
Kraft JC, Juchau MR. 9-CIS-RETINOIC ACID: A DIRECT-ACTING DYSMORPHOGEN. Biochem Pharmacol 1993;46(4):709-716.

Experiments in vitro with cultured rat conceptuses demonstrated that 9-cis-retinoic acid (9-cis-RA) (300 ng/mL amniotic fluid) produced branchial arch and somite defects similar to those elicited by equal concentrations of all-trans-retinoic acid (all-trans-RA), but with an increase in cephalic defects that included missing optic vesicles. After concept uses were intraamniotically microinjected with 600 ng 9-cis-RA/mL amniotic fluid on day 10 of gestation, an unusual heart defect was also observed. HPLC analyses indicated that 9-cis-RA readily underwent conversion to the less active metabolite, 13-cis-retinoic acid (13-cis-RA), in cultured concept uses during the first 4 hr after treatment but only after 6 hr could elevated levels of the potent dysmorphogen all-trans-RA be detected. In separate experiments, conversion of 13-cis-RA or of all-trans-RA to 9-cis-RA could not be detected during a 6-hr embryo culture period. Endogenous levels of 9-cis-RA in whole rat embryos also were below limits of detection but small quantities of this isomer could be detected in neonatal rat eye and human embryonic brain. Our present study strongly suggests that 9-cis-RA is a direct-acting dysmorphogen with probable specific

target sites of action.

88
Larson RC, Ignotz GG, Currie WB. PLATELET DERIVED GROWTH FACTOR (PDGF) STIMULATES DEVELOPMENT OF BOVINE EMBRYOS DURING THE FOURTH CELL CYCLE. Development 1992;115(3):821-6.

In vitro produced, 2-cell bovine embryos were cultured in serum-free medium supplemented with various combinations of growth factors to test the hypothesis that these polypeptide factors are able to signal preimplantation development. The developmental arrest that occurs during the 8-cell stage with typical culture methods might be relieved by a growth factor-dependent mechanism that would stimulate expression of the embryonic genome, thereby mimicking events that occur in vivo in the oviduct during the fourth cell cycle (8- to 16-cell stage). Subsequently, other growth factors might promote compaction and blastulation, processes which normally occur in the uterus. The effects of growth factors on early embryos were evaluated using phase contrast microscopy to monitor progression to the 8-cell stage, completion and duration of the fourth cell cycle, and blastocyst formation. Platelet derived growth factor (PDGF) promoted development beyond the 16-cell stage in 39.1% of the 2-cell embryos examined in all experiments. The duration of the fourth cell cycle among these embryos was approximately 26 hours. During development after the 16-cell stage, PDGF reduced the proportion of embryos bastulating from 12.7% to 5.8%; in contrast, transforming growth factor alpha (TGF alpha), acting during the same developmental time period, increased the proportion of embryos blastulating from 8.6% to 40.6%. These results, using serum-free medium, indicated that PDGF signalled completion of the fourth cell cycle. TGF alpha, and perhaps basic fibroblast growth factor (bFGF), promoted blastulation of 16-cell embryos during subsequent culture.

89
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.

90
Smoak IW. DIAZOXIDE: EMBRYOPATHIC EFFECTS OF AN ANTIHYPERTENSIVE AGENT IN VITRO AND ITS REDUCTION OF SULFONYLUREA-INDUCED DYSMORPHOGENESIS. Teratology 1993; 47(5):401-2.

Diazoxide (DZ) is an antihypertensive agent used widely for the treatment of high blood pressure and hypoglycemia, but little is known regarding its effect on embryonic development. The whole-embryo culture (WEC) method was used to expose mouse embryos for 24 hrs to DZ (0, 25, 50, 100, 200 ug/mL) at neurulating (3-6 somite) and early limb-bud (20-25 somite) stages. In addition, neurulating mouse embryos were exposed in WEC to a dysmorphogenic concentration (130 ug/mL) of the sulfonylurea, chlorpropamide (CP), to which a subteratogenic level (25 ug/mL) of DZ was added. All embryos were examined for heart rate, malformations, somite number, and total protein content. Neurulating embryos were not affected by DZ at concentrations less than or equal to 50 ug/mL but demonstrated high malformation rates at 100 ug/mL (63%) and 200 ug/mL (100%). Similarly, heart rate, somite, and protein

values were unaffected by less than or equal to 50 ug/mL DZ but were decreased by exposure to 100 and 200 ug/mL. Defects included cranial neural tube, heart, and somite abnormalities. Early limb-bud stage embryos demonstrated a similar threshold-type response to DZ, with no effect at less than or equal to 50 ug/mL and high malformation rates at 100 ug/mL (83%) and 200 ug/mL (100%). Defects at this stage included cranial neural tube, cardiovascular, and pharyngeal arch abnormalities. Exposure of neurulating embryos to 130 ug/mL CP in WEC produced a malformation rate of 70%, which was reduced to 19% by adding 25 ug/mL DZ to the culture medium. Heart rate, somite, and protein values were also improved by combined exposure to DZ and CP. Thus, DZ (greater than or equal to 100 ug/mL) produces dysmorphogenesis in neurulating and early limb-bud stage mouse embryos in WEC. In addition, a subteratogenic level of DZ reduces the embryopathic effects of the sulfonylurea CP in neurulating mouse embryos in vitro.

GENOTOXICITY

91

Skoulis NP, Barbee SJ, Jacobson-Kram D, Putman DL, San RH C. EVALUATION OF THE GENOTOXIC POTENTIAL OF ZINC PYRITHIONE IN THE SALMONELLA MUTAGENICITY (AMES) ASSAY, CHO/HGPRT GENE MUTATION ASSAY AND MOUSE MICRONUCLEUS ASSAY. J Appl Toxicol 1993;13(4):283-9.

The mutagenic potential of zinc pyrithione (Znpt) was evaluated in vitro in the Salmonella/mammalian microsome plate incorporation mutagenicity (Ames) assay and the CHO/HGPRT gene mutation assay. The clastogenic potential of Znpt was evaluated in vivo using the mouse micronucleus test. Znpt was neg. in the Ames test in five tester strains in the presence and absence of rat liver microsomal enzymes when assayed at concns. ranging between 10 and 333 mug per plate and between 0.03 and 33 mug per plate, resp. Znpt also produced neg. results in the CHO/HGPRT assay. No significant increases in mutant frequencies were seen in the presence and absence of rat liver microsomal enzymes. In each case, the highest concns. reduced cellular viability by 83% and 85%, resp. Znpt also did not induce increased frequencies of micronuclei in mouse bone marrow cells when tested at the maximally tolerated dose (MTD) (44 mg kg-1). These data support the conclusion that Znpt lacks genotoxic activity under the conditions of these tests.

92

Musk SR R, Johnson IT. IN VITRO GENETIC TOXICOLOGY TESTING OF NATURALLY OCCURRING ISOTHIOCYANATES. Spec Publ - R Soc

Chem 1993;123,(Food and Cancer Prevention: Chemical and Biological Aspects):58-61.

All three isothiocyanates (ITCs) showed significant clastogenic activity, with phenylethyl ITC generating as many as 17 aberrant cells per 100 at the D37 dose. Whilst no assays of mutagenic activity per se have been performed, the present results do indicate that these ITCs should be considered as potential carcinogens. This data contrasts with many of the published studies on allyl ITC and indeed with the authors' own observations which indicate that AITC is either nongenotoxic or at most only very weakly genotoxic, even at highly cytotoxic concns.

93
Matsuoka A, Yamazaki N, Suzuki T, Hayashi M, Sofuni T. EVALUATION OF THE MICRONUCLEUS TEST USING A CHINESE HAMSTER CELL LINE AS AN ALTERNATIVE TO THE CONVENTIONAL IN VITRO CHROMOSOMAL ABERRATION TEST. Mutat Res 1992;272(3):223-236. (REFS 13)

A method of slide preparation was developed for a micronucleus (MN) test using a Chinese-hamster lung cell line which had been routinely used forconventional chromosomal aberration (CA) testing. Tests were carried out simultaneously using the CA test on 14 clastogenic chemicals or spindle poisons which had various modes of action. The method of slide preparation for the MN test was the same as that used for the conventional metaphase analysis, except that 1% acetic-acid in methanol was the cell suspension medium for air drying. Micronuclei were induced by all chemicals tested in reproducible and dose dependent fashion. The results were in good agreement with those obtained by metaphase analysis. The authors conclude that the in-vitro MN assay using cultured mammalian cells offered a good alternative to the in-vitro CA test. The newer test methodology is simple and the observation of MN is less subjective than that of CA.

94
Gu Z-W, Whong W-Z, Wallace WE, Ong T-M. INDUCTION OF MICRONUCLEI IN BALB/C-3T3 CELLS BY SELECTED CHEMICALS AND COMPLEX MIXTURES. Mutat Res 1993;279(3):217-222. (REFS 17)

The genotoxicity of various chemicals and complex mixtures was evaluated through a study of micronuclei induction using mouse-BALB/c-3T3 cells. The chemicals tested were benzo(a)pyrene (BAP), cyclophosphamide (CPA), 2-aminoanthracene (2AA), 2-nitrofluorene (2NF). The complex

mixtures tested were cigarette smoke condensate (CSC) and nitrosated coal dust extracts (CDE). BALB/c-3T3 subclone A31-1-13 cells were seeded at a density of about 10(6) cells/dish and exposed to the chemicals for various lengths of time. Cytogenetic preparations were scored for micronuclei using 2000 interphase cells. Results showed that all tested chemicals and mixtures induced micronuclei. With BAP, CPA, 2AA and 2NF, increased frequencies of micronuclei were observed at concentrations that did not induce cytotoxicity. Except for CSC and CDE, differences between treated groups and solvent controls were significant. Both CSC and CDE were toxic at high concentrations. Dose response relationships showed correlation coefficients higher than 0.80, except for BAP which was 0.46. BAP and CPA tested for exposure times of 8 hour (hr) and 24hr, and 4hr and 8hr, respectively, showed that the longer exposure did not result in a consistent increase in induced micronuclear frequencies. The authors conclude that BALB/c-3T3 cells are capable of activating all the tested chemicals to act as procarcinogens and promutagens, and that this system may prove to be useful for screening genotoxic chemicals since the frequencies of micronuclei produced are higher than those in Chinese-hamster-V79 cells.

95
Sun TS, Stahr HM. EVALUATION AND APPLICATION OF A BIOLUMINESCENT BACTERIAL GENOTOXICITY TEST. J AOAC Int 1993;76(4):893-8.

The Mutatox test (commercial name for the bioluminescent bacterial genotoxicity test) has been shown to be a good alternative to the Ames test. The test uses dark mutants of luminous bacteria (Vibrio fischeri) and determines the ability of various genotoxic agents to restore the luminescence by inducing mutation. It provides a rapid screening test which can be used to assay the genotoxicity of large numbers of pure and complex compounds. The test is completed in 1 day, and by serially diluting the compound, dose response data plus toxicity data can be generated for a number of samples simultaneously. For the direct assay (without exogenous metabolic activation), the positive controls selected were 3,6-diaminoacridine (proflavine) and N-methyl-N-nitro- nitrosoguanidine. For the S-9 assay, which incorporated the microsome fraction (S-9) from rat liver as an exogenous metabolic activation system, the positive controls selected were aflatoxin B1 and benzo(a)pyrene. This study also indicated that methyl-imidazo-quinoline and tryptophan pyrolysates were genotoxic in the presence of S-9 activation, aflatoxin B1 epoxide and fumonisin B1 showed direct genotoxic activity,

and aflatoxin B2 and ochratoxin A were not genotoxic.

96
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 analysis of micronuclei in whole blood or separated lymphocyte cultures. The induction of binucleate cells by various concentrations of cytochalasin B (3, 4.5 or 6 micrograms/ml) was examined at two harvest times (68 or 72 h). An optimal yield was obtained by adding cytochalasin B at a dose of 6 micrograms/ml to cultures 44 h after initiation with harvest 24 h (whole blood) or 28 h (separated lymphocytes) later. Cytocentrifuge preparations of lymphocytes (separated from whole blood using commercial preparations 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 separated lymphocyte cultures, thus allowing direct comparisons of sensitivity to genotoxic agents.

97
Ashby J, Brusick D, Myhr BC, Jones NJ, Parry JM, Nesnow S, Paton D, Tinwell H, Rosenkranz HS, Curti S, et al. CORRELATION OF CARCINOGENIC POTENCY WITH MOUSE-SKIN 32P-POSTLABELING AND MUTA-RMOUSE 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.

The genotoxicities in vitro and in vivo of the mouse-skin carcinogen 7,12-dimethyl-benz[a]anthracene (DMBA) have been compared with those of its weakly carcinogenic 4,5-sulphur analogue, 6,11-dimethylbenzo[b]naphtho-[2,3-d]thiophene (S-DMBA). The only datasets that correlated with the relative carcinogenicity of these agents to the skin were those conducted using topically exposed mouse skin. Thus, both chemicals induced lacZ- mutations in the skin of lacZ+ transgenic mice, and both produced DNA adducts on mouse-skin DNA as assessed using the 32P-postlabeling technique. In each case, DMBA gave a stronger response than did S-DMBA. In contrast to these responses, only DMBA was active in the mouse bone-marrow micronucleus assay and in the C3H10T1/2 in vitro cell transformation assay. Both chemicals were

mutagenic to Salmonella and of approximately equal potency. The molecular geometry of DMBA and S-DMBA are compared, and divergent CASE predictions of activity in the Salmonella assay and skin-painting bioassay are discussed. The importance of conducting predictive genotoxicity assays in systems close to those in which carcinogenicity is to be assessed is emphasized by these data.

98
Ye SH. HYPOXANTHINE PHOSPHORIBOSYL TRANSFERASE ASSAY OF LEAD MUTAGENICITY ON KERATINOCYTES. Chung Kuo Yao Li Hsueh Pao 1993;14(2):145-7.

An improved hypoxanthine phosphoribosyl transferase (HPRT) assay system was used to investigate the genotoxicity in human and rat keratinocytes exposed to Pb2+ 0.1-100 mumol.ml-1 in vitro. Autoradiography was applied to determine the number of labeled cells/cm2 of culture with [3H]TdR and liquid scintillation spectrometry was used to determine the incorporation of [3H]TdR into DNA counting of 6-thioguanine (TG)-resistant cells. The ratio between the number of labeled cells in the Pb2+ treated group (T) and in the control group (C) was calculated. When the cells exposed to Pb2+ 6 mumol.L-1 for 4 h, the T/C ratios reached 1.75 (scintillation, S), and 2.07 (autoradiography, A) in human and 1.37 (S), and 1.77 (A) in rat cells. A positive relation existed between the concentration of Pb2+ and mutagenicity. Lead should be considered as a weak mutagen in human and rat keratinocytes.

99
Crofton-Sleigh C, Doherty A, Ellard S, Parry EM, Venitt S. MICRONUCLEUS ASSAYS USING CYTOCHALASIN-BLOCKED MCL-5 CELLS, A PROPRIETARY HUMAN CELL LINE EXPRESSING FIVE HUMAN CYTOCHROMES P-450 AND MICROSOMAL EPOXIDE HYDROLASE. Mutagen 1993;8(4):363-72.

The MCL-5 cell line is a human lymphoblastoid TK+/- cell line that constitutively expresses a relatively high level of native CYP1A1, four other human cytochromes (CYP1A2, CYP2A6, CYP3A4 and CYP2E1) and microsomal epoxide hydrolase, carried as cDNAs in plasmids. The aim of this study was to evaluate this cell line for its suitability for detecting chromosomal anomalies, employing micronucleus formation in cells blocked at cytokinesis as the indicator of clastogenicity. Results from two laboratories ('ICR' and 'Swansea') using different protocols are reported. In the ICR protocol, aflatoxin B1, sterigmatocystin, enzo[a]pyrene, dibenz[a,h]anthracene, 3-methylcholanthrene,

cyclophosphamide, N-nitrosodimethylamine, 2-amino-3,8-dimethylimidazo[4,5-f]-quinoxaline, benzidine, 2-aminofluorene, benzene, tamoxifen and omeprazole were tested and gave positive results. Anthracene, phenanthrene and pyrene were negative. In the Swansea protocol, AHH-1 cells, the parent line which constitutively expresses CYP1A1, but does not contain the genetically engineered human cytochromes or epoxide hydrolase, were tested in parallel with MCL-5 cells. Aflatoxin B1, sterigmatocystin, benzo[a]pyrene, N-nitrosodiethylamine, 2-acetylaminofluorene, benzene, omeprazole and tamoxifen were tested and gave positive results. Of these, only benzo[a]pyrene was equally potent in both cell lines. Assays of tamoxifen and omeprazole included kinetochore staining. Omeprazole, but not tamoxifen, induced a significant level of kinetochore-positive micronuclei. The detection of micronucleus formation in these genetically engineered cells appears to be a rapid, eclectic and sensitive method for screening for genotoxic activity in vitro.

100
Braun R, Huettner E, Merten H, Raabe F. GENOTOXICITY STUDIES IN SEMICONDUCTOR INDUSTRY: 1. IN VITRO MUTAGENICITY AND GENOTOXICITY STUDIES OF WASTE SAMPLES RESULTING FROM PLASMA ETCHING. J Toxicol Environ Health 1993; 39(3):309-322.

Solid waste samples taken from the etching reactor, the turbo pump, and the waste air system of a plasma etching technology line in semiconductor production were studied as to their genotoxic properties in a bacterial repair test, in the Ames/Salmonella microsome assay, in the SOS chromotest, in primary mouse hepatocytes, and in Chinese hamster V79 cell cultures. All three waste samples were found to be active by inducing of unscheduled DNA-synthesis in mouse hepatocytes in vitro. In the bacterial rec-type repair test with Proteus mirabilis, waste samples taken from the turbo pump and the vacuum pipe system were not genotoxic. The waste sample taken from the chlorine-mediated plasma reactor was clearly positive in the bacterial repair assay and in the SOS chromotest with Escherichia coli. Mutagenic activity was demonstrated for all samples in the presence and absence of S9 mix made from mouse liver homogenate. Again, highest mutagenic activity was recorded for the waste sample taken from the plasma reactor, while samples collected from the turbo pump and from the waste air system before dilution and liberation of the air were less mutagenic. For all samples chromosomal damage in V79 cells was not detected, indicating absence of clastogenic activity in vitro. Altogether, these results indicate generation of genotoxic

and mutagenic products as a consequence of chlorine-mediated plasma etching in the microelectronics industry and the presence of genotoxins even in places distant from the plasma reactor. Occupational exposure can be expected both from the precipitated wastes and from chemicals reaching the environment with the air stream.

101
Perocco P, Colacci A, Grilli S. IN VITRO CELL TRANSFORMATION INDUCED BY THE PESTICIDE FENARIMOL. Res Commun Chem Pathol Pharmacol 1993;80(3):345-356.

The pesticide fenarimol is capable of transforming BALB/c 3T3 cells in an in vitro model system, and its action resembles a carcinogenic process in vivo. In the absence of metabolic activation, transformed foci are already visible in the standard experimental procedure. The addition of the S9 fraction as an exogenous metabolic system leads to a decrement of cytotoxic effects and the reduction of the transfomation rate. The transformed phenotype, however, becomes visible when confluent cells are replated and allowed further cell replication. Transformation effects by fenarimol may be due to both a weak genotoxic activity and/or stronger promoting activity.

102
Atherholt T, Aronson M, Ginsberg G. GENETIC DAMAGE IN VITRO

IN HUMAN CELLS FOLLOWING ETHYLENE OXIDE EXPOSURE: CHROMOSOME ABERRATIONS AND DNA SINGLE STRAND BREAKS 1 AND 14 DAYS FOLLOWING EXPOSURE. Environmental Protection Agency, Washington, DC. Govt Reports Announcements & Index (GRA&I), Issue 21, 1993.

Human diploid lung fibroblast cells were exposed to various dose levels of ethylene oxide (EtO) to determine the relationship between dose, dose-rate or concentration-time effects and the formation of chromosome aberrations (CA), aneuploidy, and DNA single strand breaks (SSB) 1 day and 14 days following exposure. The chromosomal location of each CA was determined. Some of the CA experiments were also conducted with and without caffeine in the post-exposure culture medium to determine the ability of caffeine to enhance CA formation. Following 60 minute exposures with dose levels up to 10-15 mM, EtO increased the number of CA, aneuploidy, and SSB observed 20-22 hours following exposure. These effects occurred at dose levels which also resulted in some lethality and reduction in the mitotic index. Caffeine

appeared to enhance the formation of CA, but 0.25 mM caffeine did not inhibit the repair of SSB at 1 day following EtO exposure. With an assay sensitivity limit of approximately 1 percent, no detectable increase in the percentage of aberrant cells was observed 14 days following exposure. An increase in the number of aneuploid cells was observed 14 days following exposure. A small increase in SSB 14 days following exposure to 10-15 mM EtO was not considered significant. Sponsored by Environmental Protection Agency, Washington, DC.

103
Miura KF, Hatanaka M, Otsuka C, Satoh T, Takahashi H, Wakabayashi K, Nagao M, Ishidate M JR.
2-AMINO-3-METHYLIMIDAZO 4,5-F) QUINOLINE (IQ), A CARCINOGENIC PYROLYSATE, INDUCES CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER LUNG FIBROBLASTS IN VITRO.
Mutagen 1993;8(4):349-354.

The ability of 2-amino-3-methylimidazo(4,5-f)quinoline (IQ) to induce chromosomal aberrations (CAs) in Chinese hamster lung fibroblast CHL/IU) cells in vitro was examined. On incubation with rat S9 (2.5-10%, v/v) for 3 h, followed by a recovery culture period of 21 h, IQ caused significant induction of CAs at a concentration 20 mug/ml, but had less effect at 40 mug/ml. With longer recovery culture times such as 27-33 h, however, IQ was much more effective at 40 mug/ml. No significant induction was observed with 1 or 6 h treatments followed by 23 or 18 h recovery cultures, respectively. On incubation without S9, only weak CA induction by IQ was observed. These results show that IQ is a clastogen and that its clastogenic effect varied with the experimental conditions, such as the time of exposure and the time of recovery culture. The cell cycle perturbation effect is suggested to be one of the critical factors for the detection of the clastogenic potential of IQ.

104
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.

105
Ashby J, Brusick D, Myhr BC, Jones NJ, Parry JM, Nesnow S, Paton D, Tinwell H, Rosenkranz HS, et al. CORRELATION OF CARCINOGENIC POTENCY WITH MOUSE-SKIN PHOSPHORUS-32-POSTLABELING AND MUTA-MOUSE LAC Z-NEGATIVE MUTATION DATA FOR FOR DMBA AND ITS K-REGION SULPHUR ISOSTERE: COMPARISON WITH ACTIVITIES OBSERVED IN STANDARD GENOTOXICITY ASSAYS. Mutat Res 1993;292 (1):25-40.

The genotoxicities in vitro and in vivo of the mouse-skin carcinogen 7,12-dimethylbenz(alpha) anthracene (DMBA) have been compared with those of its weakly carcinogenic 4,5-sulphur analogue, 6,11-dimethylbenzo(b)-naphtho-(2,3-d)thiophene (S-DMBA). The only datasets that correlated with the relative carcinogenicity of these agents to the skin were those conducted using topically exposed mouse skin. Thus, both chemicals induced lacZ- mutations in the skin of lacZ+ transgenic mice, and both produced DNA adducts on mouse-skin DNA ass assessed using the 32P-postlabeling technique. In each case, DMBA gave a stronger response than did S-DMBA. In contrast to these responses, only MBA was active in the mouse bone-marrow micronucleus assay and in the C3H10T1/2 in vitro cell transformation assay. Both chemical were mutagenic to Salmonella and of approximately equal potency. The molecular geometry of DMBA and S-DMBA are compared, and divergent CASE prediction of activity in the Salmonella assay and skin-painting bioassay are discussed. The importance of conducting predictive genotoxicity assays in systems close

to those in which carcinogenicity is to be assessed is emphasized by these data.

106
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):317-320.

A modified protocol is described for the in vitro analysis of micronuclei in whole blood or separated lymphocyte cultures. The induction of binucleate cells by various concentrations of cytochalasin B (3, 4.5 or 6 mug/ml) was examined at two 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 (separated lymphocytes) later. Cytocentrifuge preparations of lymphocytes (separated from whole blood using commercial preparations 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 separated lymphocyte cultures, thus allowing direct comparisons of sensitivity to genotoxic agents.

ORGAN CULTURE
107
Amacher DE, Stadler J, Schomaker SJ, Verseil C. POSSIBLE DEVELOPMENTAL EFFECTS OF SOME ARYL TRIAZINE ANTICOCCIDIAL AGENTS IN RAT LIMB BUD MICROMASS CULTURES AND IN RAT EMBRYOCULTURE. Teratology 1992;46(3):19A.

When cultured at high density, limb bud mesenchyme cells isolated from 13 day rat embryos proliferate and differentiate into chondrocytes providing an in vitro model for early skeletal development. In this system, suspected teratogens diminish cartilage proteoglycan synthesis thus inhibiting cell differentiation, an effect predictive of teratogenicity in vivo (Flint & Orton, Toxicol. & Appl. Pharmacol. 76:383, 1984). In this study the amount of drug required to cause a 50% inhibition of alcian blue uptake (PG50) by cartilage proteoglycans in spot cultures of limb bud cells was used to assess teratogenic potential in vitro following 48 hour exposure to each of 4 anticoccidials, 3 metabolites, and, for comparison, 6-azauracil. Following drug removal, cultures were incubated another 96 hours, then cells were fixed and stained with 0.5% alcian blue.

Bound dye was extracted and quantitated. In parallel cultures, cell viability was measured by neutral red uptake and protein content was assayed by the BCA method. Test concentrations were usually 5-250 ug/mL. PG50 values less than 50 ug/mL were obtained for CP-25,415 and its metabolite CP-25,641; CP-30,542 and its metabolites CP-37,537 and CP-32,107; and CP-25,722 suggesting potential teratogenicity as described by Flint (Fd. Chem. Toxic. 24:627, 1986). PG50 values for CP-21,745 and 6-azauracil were greater than 250 ug/mL suggesting no teratogenic potential. Both CP-32,107 and CP-25,722 were considerably less cytotoxic than the other 4 in vitro proteoglycan synthesis inhibitors, suggesting greater potency as developmental toxicants. When 3 of these compounds (CP-25,722, CP-25,415 and CP-30,542) were tested in a 48-hour rat embryoculture model, dysmorphogenicity was evidenced for all of them. CP-25,722 induced developmental defects at 30 ug/mL, a dose which was not embryotoxic. CP-25,415 and CP-30,542 produced these defects only at higher concentrations, in the presence or absence of embryotoxicity, respectively. The potency of CP-25,722 as a developmental toxicant has been confirmed in vivo where it was teratogenic in rats at a dose level which was devoid of any other embryo- or fetotoxicity and in the absence of any maternal toxicity. The absence of teratogenic potential of CP-21,745 in the limb bud micromass culture has been also confirmed in an in vivo study in rats.

108
Repetto M, Halters S, Schowing J. THE ACTION OF AY9944 UPON THE EARLY DEVELOPMENT OF THE CHICK EMBRYO CULTIVATED IN-VITRO. 21st Annual Conference of the European Teratology Society, Lyon, France, September 6-9, 1993. Teratology 1993;48(2):33A.

No abstract.

METABOLISM/XENOBIOTICS
109
Dogterom P, Rothuizen J. A SPECIES COMPARISON OF TOLBUTAMIDE METABOLISM IN PRECISION-CUT LIVER SLICES FROM RATS AND DOGS. QUALITATIVE AND QUANTITATIVE SEX DIFFERENCES. Drug Metab Dispos Biol Fate Chem 1993;21(4):705-9.

Precision-cut liver slices from rats and dogs were used to investigate in vitro the metabolism of tolbutamide. Tolbutamide (100 microM) was incubated with liver slices in 12-well plates (rat: 1 slice/well; dog: 4 slices/well) for up to 9 hr. In rats, ualitative sex differences were found in tolbutamide metabolism, whereas in dogs quantitative sex

differences were found. In male and female rats, the major metabolite was hydroxytolbutamide, with minor amounts of carboxytolbutamide. In both sexes, the formation rates of these metabolites were the same. In male rats, also the "dog-specific" metabolites, p-tolylsulfonylurea and p-tolylsulfonamide, were found. No direct toxicity of tolbutamide and its metabolites was observed. In male and female dogs, the major metabolite was p-tolylsulfonylurea, with smaller amounts of hydroxy- and carboxy-tolbutamide. Formation rates of the various tolbutamide metabolites in male dogs were approximately 3 times higher than in female dogs. The "dog-specific" metabolite p-tolylsulfonamide could not be detected. These results show that precision-cut liver slices are capable of detecting hitherto unknown species and sex differences in the metabolism of tolbutamide. Liver slices are a promising in vitro method for the study of comparative drug metabolism.

110
Dogterom P. DEVELOPMENT OF A SIMPLE INCUBATION SYSTEM FOR METABOLISM STUDIES WITH PRECISION-CUT LIVER SLICES. Drug

Metab Dispos Biol Fate Chem 1993;21(4):699-704.

The use of precision-cut liver slices for toxicity and drug metabolism studies becomes more and more popular. So far, most of these studies are conducted using the dynamic organ culture system as incubation system. However, this system has some disadvantages, especially for the study of drug metabolism. Therefore, the aim of this study was to develop a simple incubation system for precision-cut liver slices. Rat liver slices were incubated individually in 12-well culture plates filled with 1.5 ml Krebs-Henseleit buffer, pH 7.4. Instead of glucose, fructose was used as carbohydrate source. The plates were put on a gyratory shaker (90 rpm) in a temperature controlled incubator (37 degrees C) under an atmosphere of 95% air/5% CO2. Under these conditions slices could be kept viable for at least 11 hr, which seems to be long enough for metabolism studies. Slice thickness was a critical factor in both studies on optimal incubation conditions and metabolism studies. A correlation was found between slice thickness (i.e. slice weight) and metabolite production (amount formed/mg slice) as demonstrated with tolbutamide and diazepam as test substances. It is demonstrated that a variation in slice thickness does not alter the number of cells involved in drug metabolism (i.e. the absolute amount of metabolite formed per slice does not alter). In conclusion, the way liver slices are incubated as well as the thickness of slices highly determines the results of studies on incubation conditions and metabolism

studies with precision-cut liver slices.

111
Kokkinakis DM, Reddy MK, Norgle JR, Baskaran K. METABOLISM AND ACTIVATION OF PANCREAS SPECIFIC NITROSAMINES BY PANCREATIC DUCTAL CELLS IN CULTURE. Carcinogenesis (OXF) 1993; 14(8):1705-1709.

Metabolism of 14C labeled N-nitrosobis(2-oxopropyl)amine (BOP), N-nitroso(2-hydroxypropyl) (2-oxopropyl)amine (HPOP) and N-nitrosobis(2-hydroxypropyl)amine (BHP) by pancreatic duct cells in culture involves the following two pathways: reduction or oxidation reactions at the beta-carbon which result in the inter-conversion of these nitrosamines and activation reactions which result in the decomposition of the nitrosamine, the evolution of 14CO2 and the labeling of macromolecules. Reduction of BOP to HPOP seems to contribute significantly to the metabolism of the former nitrosamine by pancreatic duct cells, however, redox reactions at the beta-carbon of HPOP or BHP are not extensive. In terms of DNA damage, all three nitrosamines yield methyl and hydroxypropyl adducts. As expected, HPOP and BHP yield higher levels of O6-hydroxypropylguanine than BOP, while the latter yields higher levels of O6-methylguanine. There is no correlation between the ability of these nitrosamines to alkylate duct cell DNA in vitro and their carcinogenic potency in vivo. Concentrations of DNA adducts induced by pancreas specific nitrosamines (PSNs) in cultured duct cells at concentrations comparable to those found in the pancreatic juice of animals treated with BOP, are almost an order of magnitude lower than those induced in the pancreas of such animals. Discrepancies between in vitro and in vivo formation of active metabolites and DNA adducts may be attributed to the decline of the cells' ability to activate PSNs during culturing. In the saline vein, the ductal cell may not be the main source of active metabolites targeting its DNA in the animal model.

112
Ademola JI, Wester RC, Maibach HI. ABSORPTION AND METABOLISM OF 2-CHLORO-2,6-DIETHYL-N-(BUTOXYMETHYL)ACETANILIDE (BUTACHLOR) IN HUMAN SKIN IN VITRO. Toxicol Appl Pharmacol 1993;121(1):78-86.

Studies have demonstrated that several chemicals are absorbed and metabolized during skin permeation. We investigated the absorption and metabolism of the pesticide butachlor. Radiolabeled butachlor was measured in human (n =

5) skin and the unchanged compound and metabolites were quantified by high-pressure liquid chromatography (HPLC) and thin-layer chromatography (TLC). Following a 24-hr exposure, an average butachlor quantity of 5.00% of the applied dose (1.01 mug) was absorbed by the skin. The mean peak penetration rate was 0.7% of the applied dose per hour. The skin retained 1.40 to 8.10% of the applied butachlor. The retention of 1.4 to 8.1% of the pesticide by the skin suggests the importance of monitoring human skin following topical exposure. Of the dose recovered in the skin, 0.9% was metabolized to 4-hydroxybutachlor, while 1.8% of the dose in the receptor fluid was recovered as polar conjugates (cysteine, 0.29% dose; glutathione, 0.1% dose; unidentified metabolites, 1.4% dose); 2.8 and 6.8% of the dose absorbed by the skin (5.0%) were recovered as metabolites in the receptor fluids and skin homogenates, respectively. Similar to metabolism during percutaneous absorption, butachlor was metabolized to its conjugated and hydroxyl derivatives by skin fractions. The rate of butachlor glutathione and butachlor cysteine formation using skin cytosolic fractions were 12.0:1.5 and 48.0: 3.6 pmol/min/mg protein: SD, respectively. When human skin microsomes were incubated with butachlor, 4-hydroxybutachlor was formed at the rate of 55.0:15.0 pmol/min/mg protein : SD. 4-Hydroxybutachlor formation was totally dependent on the presence of NADPH. The biotransformation of butachlor using skin fractions indicates the metabolic capacity of the tissue. The biological significance of these metabolites in the deposition of butachlor requires further investigation.

MODELING SYSTEM/TOXICOLOGICAL
113
Sundman-Engberg B, Tidefelt U, Gruber A, Paul C.
INTRACELLULAR CONCENTRATIONS OF MITOXANTRONE IN LEUKEMIC CELLS IN VITRO VS IN VIVO. Leuk Res 1993;17(4):347-52.

The aim of this study was to determine the intracellular pharmacokinetics of mitoxantrone in vivo and to use these results to establish how leukemic cells should be incubated to perform clin. relevant in vitro studies of this drug. Blood samples were obtained from 11 patients with acute nonlymphoblastic leukemia at certain intervals up to 20 h after the infusion of mitoxantrone 12 mg/m2. Plasma and leukemic cells were separated and the drug concns. were determined with HPLC. Before treatment, leukemic cells from 12 patients were incubated with 0.02, 0.05, 0.1, 0.2 and 1.0 muM mitoxantrone for 1-4 h and thereafter cultured in suspension culture for 20 h; during this time cell samples were taken at certain intervals for drug detn. In cells incubated with 0.05 and 0.2 muM mitoxantrone the cytotoxic

effect was measured with the Disk assay after cultivation for 4-5 days. In vivo, the intracellular levels exceeded the plasma concns. already at the end of infusion and after 2 h the intracellular concns. were 200-300 times higher than in plasma. In vitro, the intracellular steady state level of mitoxantrone was reached after 1-2 h and there was apronounced intracellular retention even after 20 h culture in drug-free medium. Incubation with 0.05 muM during 1 h gave intracellular concns. of mitoxantrone similar to those achieved in vivo. This incubation concentration gave a mean cytotoxic effect of 53% living cells measured with the Disk assay, which gives good possibilities to discriminate between mitoxantrone- sensitive and unsensitive cells. Thus, exposing leukemic cells in vitro for in vivo mimicking mitoxantrone concentrations could increase the clinical relevance of predictive assays.

114
Clarke DO, Elswick BA, Welsch F, Conolly RB. PHARMACOKINETICS OF 2-METHOXYETHANOL AND 2-METHOXYACETIC ACID IN THE PREGNANT MOUSE: A PHYSIOLOGICALLY BASED MATHEMATICAL MODEL. Toxicol Appl Pharmacol 1993; 121(2):239-252.

A physiologically based pharmacokinetic (PBPK) model was created to describe the disposition of 2-methoxyethanol (2-ME) and its teratogenic metabolite, 2-methoxyacetic acid (2-MAA), in the pregnant CD-1 mouse. The model's foundation is a mathematical description of the physiological changes that occur during gestation (O'Flaherty et al., Toxicol. Appl. Pharmacol. 112, 245-256, 1992). The PBPK model was developed and validated with data collected in Gestation Day (GD) 11. Absorption, distribution, and oxidation of 2-ME to 2-MAA and ethylene glycol (EG) were simulated. Flow-limited disposition of 2-ME in maternal tissues was described using in vitro-determined tissue partition coefficients (PCs). The maximum velocity (Vmax) of 2-ME oxidation to 2-MAA was calculated from literature-based in vitro data. Vmax for EG formation, and Michaelis constants for 2-MAA and EG pathways, were estimated from optimized simulations of plasma 2-ME and metabolite levels obtained after intravenous injection of 5-600 mg 2-ME kg-1. 2-MAA disposition and elimination in the dam were described by a nonphysiological one-compartment model, which was linked to the 2-ME model, based on the volume of distribution (0.510 litersverall elimination rate constant (0.124 hr-1) calculated from iv 2-MAA plasma concentration-time courses. Transfer of 2-MAA between the placenta and conceptus was described as a diffusion-limited process to more accurately simulate the higher concentrations of 2-MAA determined in

embryonic compartments compared with maternal plasma levels. Subsequent 2-MAA disposition within the embryo proper and surrounding fluid of the GD 11 conceptus was adequately described using embryo/blood (0.94) and extraembryonic fluid/blood (1.33) PCs. Extension of the PBPK model to oral and subcutaneous 2-ME administrations required optimization of first-order absorption rates; model simulations agreed closely with measured 2-ME/2-MAA levels. With refinements and further validation, the PBPK model of 2-ME/2-MAA disposition should prove helpful for extrapolation throughout gestation and between species.

115
Clewell H J III. COUPLING OF COMPUTER MODELING WITH IN-VITRO METHODOLOGIES TO REDUCE ANIMAL USAGE IN TOXICITY TESTING. 1992 Toxicology Conference on Applications of Advances in Toxicology to Risk Assessment, Wright-Patterson Air Force Base, Ohio, USA, May 19-21, 1992. Toxicol Lett (AMST) 1993;68(1-2):101-117.

No abstract.

116
Adickes ED, Mollner TJ, Makoid MC. COMPUTER-ASSISTED MATHEMATICAL CLARIFICATION OF AN IN-VITRO FAS MODEL. Research Society of Alcoholism Meeting, San Antonio, Texas, USA, June 19-24, 1993. Alcohol Clin Exp Res 1993;17(2):486.

No abstract.

117
KREWSKI D, LEROUX BG, BLEUER SR, BROEKHOVEN LH. MODELING THE AMES SALMONELLA MICROSOME ASSAY. Biometric 1993; 49(2):499-510.

Of the many short-term tests for mutagenicity that have been proposed in recent years, the Ames Salmonella/microsome assay is the single most widely used and most thoroughly validated in vitro test system. This assay uses cells cultured in a soft agar containing a trace amount of histidine to allow growth of auxotrophic bacteria, and is designed to detect reverse mutations from auxotrophic cells to histidine-independent prototrophic cells. In this paper, statistical models that have been proposed for the analysis of Ames test data are reviewed, including those of a mechanistic and empirical nature. An extension to the class of biologically based models derived by Margolin, Kaplan, and Zeiger (1981, Proceedings of the National Academy of

Sciences 78, 3779-3783) is proposed by allowing for diffusion of histidine within the plate agar. Quasi-likelihood methods for estimating the model parameters are presented, and applied to 1,120 data sets from a recent collaborative trial sponsored by the International Programme on Chemical Safety.

MUTAGENICITY

118

Ni Z, Li S, Liu Y, Tang Y, Pang D. INDUCTION OF MICRONUCLEUS BY ORGANOPHOSPHORUS PESTICIDES BOTH IN VIVO AND IN VITRO. Huaxi Yike Daxue Xuebao 1993;24(1):82-6.

A total of 22 organophosphorus pesticides (OPPs), including 8 ethyl-, 9 methyl-, and 5 other OPPs, were tested for mutagenicity in micronucleus assay system both in 615 mouse marrow cells in vivo with multi-i.p. administrations and in cultured Chinese hamster lung (CHL) cells in vitro. The structure-mutagenicity relationship of OPPs was examd. Among the OPPs tested in vivo, 5 ethyl-OPPs (diazinon, chlorpyrifos, disulfoton, ethion, and parathion), and only 1 methyl-OPP (dimethoate) were mutagenic. The other OPPs were neg. Six ethyl-OPPs (azinphos Et, chlorpyrifos, ethion, parathion, phosalone, and quinaphos), 8 (methyl-OPPs) (azinphos Me, chlorpyrifos Me, dichlorvos, dimethoate, fenitrothion, malathion, parathion Me, and tri-Me phosphate), and 2 other OPPs induced micronucleus in CHL cells in vitro. Evidently, most of the ethyl-OPPs tested showed the ability to induce micronucleus both in vivo and in vitro, and that most of the methyl-OPPs were pos. only in vitro. The mechanism of the mutagenic activity in micronucleus assay in vivo and in vitro produced by different kinds of OPPs is also discussed.

119

Ni Z, Li S, Liu Y, Tang Y, Pang D. INDUCTION OF MICRONUCLEUS BY ORGANOPHOSPHORUS PESTICIDES BOTH IN VIVO AND IN VITRO. Hua Hsi I Ko Ta Hsueh Hsueh Pao 1993;24(1):82-6.

A total of 22 organophosphorus pesticides (OPPs), including 8 ethyl-, 9 methyl-, and 5 other OPPs, were tested for mutagenicity in micronucleus assay system both in 615 mouse marrow cells in vivo with multi-intraperitoneal administrations and in cultured Chinese hamster lung (CHL) cells in vitro; and structure-mutagenicity relationship of OPPs was analyzed. Among the OPPs tested in vivo, 5 ethyl-(diazinon, chlorpyrifos, disulfoton, ethion, and parathion), and only 1 methyl-(dimethoate) were found mutagenic, while the other OPPs were negative. Six ethyl-

(azinphos ethyl, chlorpyrifos, ethion, parathion, phosaione, and quinaphos), 8 methyl- (azinophos methyl, chlorpyrifos methyl, dichlorvos, dimethoate, fenitrothion, malathion, parathion methyl, and trimethyl phosphate), and 2 other OPPs (EII and MIA), however, induced micronucleus in CHL cells in vitro. The results indicated that most of the ethyl-OPPs tested showed the ability to induce micronucleus both in vivo and in vitro, and that most of the methyl-OPPs were positive only in vitro. The mechanism for the adversity of mutagenic activity in micronucleus assay in vivo and in vitro produced by different kinds of OPPs was also discussed.

120
Kulka U, Paul D, Bauchinger M. DEVELOPMENT OF SHORT-TERM MUTAGENICITY TEST SYSTEMS IN VITRO: METABOLIC ACTIVATION OF INDIRECTLY ACTING MUTAGENS BY THREE IMMORTAL RAT HEPATOCYTE LINES. Mutagenesis 1993;8(3):193-197.

The metabolic capacity to activate the indirectly acting promutagens aflatoxin B1, cyclophos-phamide, benzo(a)pyrene, 7,12-dimethylbenz(a)anthracene and dimethylnitrosamine into DNA-reactive metabolites was investigated in three immortalized rat hepatocyte cell lines (NRL cl-B, NRL cl-C and ARL) by analyzing chromosome aberrations and sister chromatid exchange (SCE). In all three cell lines a significant clastogenic and SCE inducing response was observed after exposure to each test compound. Furthermore, activities of the two enzymes aryl hydrocarbon hydroxylase and aldrinepoxidase, which play major roles in the cytochrome P450-dependent metabolism, could be determined in all cell lines. In contrast to the hepatocyte lines in V79 Chinese hamster cells, which were used as a reference cell line without any cytochrome P450 metabolizing capacity, no arylhydrocarbon hydroxylase or aldrinepoxidase activities were detected. A cytogenetic response to the test compounds was only observed in the presence of the exogenous activating system S9 mix. Due to the wide, efficient and stable spectrum of their metabolizing capacities, the tested rat hepatocyte lines offer promising perspectives as alternative assay systems for the detection of indirectly acting mutagens.

NEPHROTOXICITY
121
Kays SE, Berdanier CD, Swagler AR, Lock EA, Schnellmann RG. AN IN VITRO MODEL OF RENAL PROXIMAL TUBULE CELL REGENERATION. J Pharmacol Toxicol Methods 1993; 29(4):211-215.

The ability of renal cells to regenerate is critical for the recovery of renal function following injury. Research on the recovery of renal function has been limited by the lack of in vitro models of renal repair. The goal of this study was to develop an in vitro model of renal proximal tubule cell (RPTC) injury and regeneration using primary cultures of rabbit RPTC. Renal proximal tubules were isolated and cultured in hormonally defined DME/F-12 medium at 37~ C under 95% air/5% CO2 RPTC were grown to confluency, made quiescent by the removal of insulin and hydrocortisone from the medium for 24-48 hr, and treated with the nephrotoxicant, 1,2-dichlorovinyl-L-cysteine (DCVC). DCVC (100 muM for 2 hr, n = 3-6) resulted in cell injury and the release of nonviable cells from the plate at 24 hr (55% : 6% confluency, mean : SEM) and 48 hr (37% : 7% confluency). Cell monolayers began to regenerate 96 hr after exposure (57% : 9% confluency) and continued to regenerate reaching 76% : 8% and 84% : 1% confluency by 6 and 8 days postexposure. Control cells maintained confluency throughout the experiment. Thus, an in vitro primary cell culture model has been developed in which the cell monolayer regenerates after nephrotoxicant-induced injury. This model may be useful in the study of mechanisms of renal cell injury and repair.

NEUROTOXICITY

122

Jones HB, Pillar AM, Prince AK. MORPHOLOGICAL ASSESSMENT OF ETHYL CHOLINE MUSTARD AZIRIDINIUM-INDUCED NEUROTOXICITY IN RAT BRAIN REAGGREGATE CULTURES. ACTA Neuropathol 1993; 86(2):154-162.

Foetal rat brain reaggregate cultures have been employed to investigate the morphological changes associated with the neurotoxic action of ethyl-choline mustard azifidinium (ECMA). In a companion study we provided evidence for apparent selective cholinergic neurotoxicity. Exposure of 9-day-old cultures to 12.5 muM ECMA for 3 days produced dilatation of selected axon preterminals and terminals in the outer core tissue layer. Axoplasm in these dilated terminals was electron lucent and contained a flocculent, plasma-like material with remnants of the smooth endoplasmic reticulum. Their synaptic vesicle content was much reduced or, absent. Microglial cells were engaged in phagocytosis of these effete structures and a few necrotic neurons were enveloped by glial processes. Exposure to 50 muM ECMA produced widespread necrosis with some surviving neurons, surrounded by the still-persisting capsular layer. Treatment with 100 muM ECMA generated a greater extent of tissue

necrosis, with only a few surviving neurons and glial cells being contained within the necrotic tissue mass. Reaggregates frequently disintegrated following capsule loss. Our results indicate that the initial morphological manifestation of ECMA-induced toxicity is dilatation of axon terminals, that are probably of cholinergic origin and are targeted due to their possession of the high-affinity choline transport system which is unique to these neurons.

123
Abdulla EM, Campbell IC. USE OF NEURITE OUTGROWTH AS AN IN-VITRO METHOD OF ASSESSING NEUROTOXICITY. Johannessen, J. N. Annals of the New York Academy of Sciences, 679 Markers of Neuronal Injury and Degeneration; Conference, Bethesda, Maryland, USA, April 22-24, 1992. XII+432P. New York Academy of Sciences: New York, New York, USA. ISBN 0-89766-796-4 (PAPER); ISBN 0-89766-795-6(CLOTH);0(0) 1993. 276-279.

No abstract.

124
Abdulla EM, Campbell IC. IN VITRO TESTS OF NEUROTOXICITY. J Pharmacol Toxicol Methods 1993;29(2):69-75. (REFS 31)

Some of the newer techniques in the rapidly advancing area of neurotoxicity testing in vitro. In vitro testing offers the possibility of relatively inexpensive screening of large numbers of pharmaceutical compounds, formulations, and environmental substances. The level of sophistication attained in this field may soon allow much more accurate safety limits to be set, as specific mechanisms of neurotoxicity are elucidated.

125
Muller J, Bruinink A, Schlatter C. NEUROTOXICITY OF ALUMINIUM ON EMBRYONIC CHICK BRAIN CULTURES. Experientia 1992;48(Abstr):A90.

Toxic damage of brain cells by aluminium (Al) is discussed as a possible factor in the development of the senile and presenile dementia of the Alzheimer type in humans. In order to investigate the sensitivity of various brain tissues to Al, serum-free cultures of mechanically dissociated embryonic chick (stage 28-29) brainstem, forebrain and optic tectum and for comparison meninges cells were prepared. Cultures were treated with Al (0-1000 uM AlCl3) from day 1 to 8 in vitro. On day 8 viability (MTT- and NR-assay) and differentiation (expression of microtubule-associated

protein type 2, glial fibrillary acidic protein and neurofilament 68kD antigens) were measured. Results suggest that differentiation and viability expressed as IC50 were influenced at about 10 times lower concentrations in cultures of optic tectum (IC50: around 100 uM Al) than necessary to affect the other cultures tested.

126
Nostrandt AC, Ehrich M. MODIFICATION OF MIPAFOX-INDUCED INHIBITION OF NEUROPATHY TARGET ESTERASE IN NEUROBLASTOMA CELLS OF HUMAN ORIGIN. Toxicol Appl Pharmacol 1993; 121(1):36-42.

A neuroblastoma cell line of human origin was used as an in vitro model system to examine early effects on inhibition of neuropathy target esterase (NTE, also known as neurotoxic esterase) in the presence of agents belonging to classes of chemicals previously demonstrated to modify organophosphorus-induced delayed neuropathy in hens. For this study, differentiated SY-5Y cells were treated for up to 10 min with mipafox, an organophosphorus compound, and NTE inhibition was determined when cells exposed to mipafox were also exposed to the carbamate, aldicarb, and to the calcium channel blocker, verapamil. Cells were exposed to aldicarb or verapamil 5 min before, at the same time, or 2 min after mipafox. Less NTE inhibition was observed when either aldicarb or verapamil was included in the incubation of SY-5Y cells with mipafox. Effects of aldicarb and verapamil on NTE inhibition in differentiated SY-5Y cells were similar to effects in chicken brain homogenates. These results indicate that NTE inhibition can be detected in neuroblastoma cells, that these cells respond in a manner similar to chicken brain, and that mipafox-induced inhibition of NTE can be decreased in the presence of aldicarb or verapamil.

127
Abdulla EM, Campbell IC. USE OF NEURITE OUTGROWTH AS AN IN-VITRO METHOD OF ASSESSING NEUROTOXICITY. Johannessen, J. N. Annals of the New York Academy of Sciences, Vol. 679. Markers of Neuronal Injury and Degeneration; Conference, Bethesda, Maryland, USA, April 22-24, 1992. XII+432P. New York Academy of Sciences: New York, New York, USA. ISBN 0-89766-796-4(PAPER); ISBN 0-89766-795-6(CLOTH); 0(0). 1993. 276-279.

No abstract.

128
Veronesi B, Ehrich M. USING NEUROBLASTOMA CELL LINES TO EXAMINE ORGANOPHOSPHATE NEUROTOXICITY. In Vitro Toxicology. A Journal of Molecular and Cellular Toxicology;6(1):57-65. (REFS 35)

The use of human and animal neuroblastoma cell lines for the evaluation of the neurotoxicity of organophosphates was studied. Three human and several mouse cell lines were maintained in culture, exposed to various organophosphates, and evaluated using the neutral red cytotoxicity assay and an esterase inhibition assay. The baseline activity of carboxylesterase (CbxE) was higher than that of neurotoxic-esterase (NTE) and the activities of cholinesterase, CbxE, and NTE were higher in human cells compared with mouse cells. Cytotoxicity studies on the SY5Y-human and NB41A3-mouse neuroblastoma cell lines demonstrated that the mouse cells were more sensitive to the tested compounds than the human cells. The differences in cytotoxic sensitivity were more pronounced to the metabolically dependent insecticide protoxicant parathion than to its metabolically active compound paraoxon. A significantly higher degree of NTE inhibition was seen following treatment with agents that were capable of producing organophosphate induced delayed neuropathy (OPIDN) in-vivo such as diisopropylfluorophosphate and mipafox, compared with other organophosphates that did not produce OPIDN such as parathion and paraoxon.

OCULARY TOXICITY
129
Decker D, Stemp M, Harper R. EVALUATION OF THE EYTEX SYSTEM FOR USE AS A PREDICTOR OF OCULAR IRRITANCY: II. HAIR CONDITIONERS AND HAIR SPRAYS. J Toxicol, Cutaneous Ocul Toxicol 1993; 12(4):377-86.

The Eytex in vitro assay was used to evaluate 33 opaque and clear conditions and 48 styling aids including hair sprays, mousses, styling gels, and lotions. The assay, which operates on the principle of protein pptn., is quant. and relatively inexpensive. Two different protocols were used: the rapid membrane assay (RMA) protocol for the conditions, mousses, styling gels, and lotions; and the upright membrane assay (UMA) for the hair sprays. All samples were tested in two or more sep. expts. and the scores averaged. One hundred percent of the conditions, 91% of the hair sprays, 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 conditions, 0.14 for the clear conditions, 0.87 for hair sprays, and 1.0 for the other styling aids. The Eytex in vitro assay for ocular irritancy can be highly predictive of Draize eye scores for opaque conditions and styling aid products. Such assays could be useful as a screening tool in new product development.

130
Kristen U, Hoppe U, Pape W. THE POLLEN TUBE GROWTH TEST: A NEW ALTERNATIVE TO THE DRAIZE EYE IRRITATION ASSAY. J Soc Cosmet Chem 1993;44(3):153-62.

The pollen tube growth test (PTG test), recently developed to detect the cytotoxicity of bioactive chemicals, is compared with the well-established Draize eye irritation assay. The PTG test, based on the growth response of in vitro growing tobacco pollen tubes, is described in detail. Comparison of the corresponding growth response data of both assays, using 22 surfactants as test substances, reveals highly significant rank correlations ($r = 0.73$, $p = 0.0018$). In both assays, the most toxic substances are among the group of anionic sulfate/sulfonate surfactants, whereas the nonionic surfactants show moderate effects. The results of the comparison clearly demonstrate that the PTG test is well suited to screen irritation potentials of surfactants and provides reliable results with good reproducibility. Moreover, the PTG test is inexpensive and helps to reduce or even avoid painful animal testing in this application.

131
Li X, Beebe DC. TRANSCRIPTIONAL CONTROL OF DELTA-CRYSTALLIN GENE EXPRESSION IN THE CHICKEN EMBRYO LENS: DEMONSTRATION BY A NEW METHOD FOR MEASURING MRNA METABOLISM. Mol Cell Biol 1993; 13(6):3282-90.

Crystallins are proteins that accumulate to very high concns. in the fiber cells of the lens of the eye. Crystallins are responsible for the transparency and high refractive index that are essential for lens function. In the chicken embryo, delta-crystallin accounts for more than 70% of the newly synthesized lens proteins. D. labeling and gene-specific polymerase chain reaction (PCR) were used to det. the mechanism regulating the expression of the two very similar delta-crystallin genes. Newly synthesized RNA was sepd. from preexisting RNA by incubating the lenses with 15N- and 13C-labeled ribonucleosides and then sepg. newly synthesized, d.-labeled RNA from the bulk of light RNA by equil. d.

centrifugation in NaI-KI gradients. The relative abundances of the two crystallin mRNAs in the sepd. fractions were then detd. by PCR. This method permitted the quantitation of newly synthesized processed and unprocessed delta-crystallin mRNAs. Additional studies used intron- and gene-specific PCR primers to determine the relative expression of the two delta-crystallin genes in processed RNA and unprocessed RNA extd. from different regions of the embryonic lens. Results of these tests indicated that the differential expression of the delta-crystallin genes was regulated primarily at the level of transcription. This outcome was not expected on the basis of the results of previous studies, which used in vitro transcription and transfection methods to evaluate the relative strengths of delta-cryst. promoter and enhancer sequences. The data suggest that the cultured cells used in these earlier studies may not have provided an accurate view of delta-crystallin regulation in the intact lens.

132
Torracca MT, Biagi A, Perini G, Saettone MF. PREPARATION AND IN VITRO AND IN VIVO EVALUATION OF INDOMETHACIN-CONTAINING OPHTHALMIC VEHICLES. Boll Chim Farm 1993;132(2):41-2.

Ophthalmic solns. and inserts were prepd. from indomethacin meglumine salt, indomethacin arginine salt, and a complex of indomethacin with the terpolymer vinylcaprolactam-vinylpyrrolidone-dimethylaminoethyl methacrylate. The inserts were tested for their ability to release indomethacin in vitro, and both the solns. and inserts were tested for ability to inhibit inflammation of the rabbit eye. In the latter test, the inserts at one-sixth the dose of the solutions produced a comparable anti-inflammatory effect.

133
Tsuchiya T, Arai T, Ohhashi J, Imai K, Kojima H, Miyamoto S, Hata H, Ikarashi Y, Toyoda K, et al. RABBIT EYE IRRITATION CAUSED BY WEARING TOXIC CONTACT LENSES AND THEIR CYTOTOXICITIES: IN VIVO/IN VITRO CORRELATION STUDY USING STANDARD REFERENCE MATERIALS. J Biomed Mater Res 1993;27(7):885-93.

To clarify the relationship between eye irritancy and cytotoxic potential induced by irritant materials, the authors made lenses coated with std. ref. materials (SRMs) prepd. from various amts. of zinc diethyldithiocarbamate (ZDEC) and polyurethane (PU). ZDEC was classified as a mild irritant by the Draize eye irritation test. When ZDEC-SRM coated contact lenses were applied to rabbit eyes, Draize scores increased in proportion to both the ZDEC and PU

concetrations used for coating. Furthermore, correlation with the cytotoxic potential (.apprx. = -0.93) was better than with lactate dehydrogenase (LDH) activities of tears from rabbit eyes wearing these coated lenses (.apprx. = 0.78). In conclusion, in vivo eye irritancy induced by wearing lenses could be established quant. with the cytotoxic potentials using a colony assay. Furthermore, the authors could compare different sensitivities caused by the same set of SRMs among three different sites of tissue. As a result, the order of sensitivity was eye > muscle > skin.

134
Osborne R, Perkins MA, Roberts DA. IN VITRO MODEL FOR EYE AND SKIN IRRITATION TESTING. PCT Int. Appl. PATENT NO. 93 17336 09/02/93 (Procter and Gamble Co.)

A technique is disclosed for testing of ocular and dermal irritants. The process involves the topical application of, liq., solid, granular, or gel-like materials (e.g. cosmetics) to a cell culture and then evaluating the cytotoxicity of the material. Cell cultures of human skin without a stratum corneum and having a histol. similarity to the eyes are used. Irritation is evaluated by measuring cell viability using an MTT assay (based on the redn. of a tetrazolium dye by functional mitochondria) or by testing for release of LDH or PGE2. A unique method of applying test materials which are not water-sol. is also described. The methodol. of the invention was applied to strong (e.g. NaOH, dish detergent), mild to moderate (e.g. laundry detergent, skin care cream), and innocuous to slight (e.g. liq. fabric softener,toothpaste) irritant test materials.

135
Ikarashi Y, Tsuchiya T, Nakamura A. COMPARISON OF THREE IN VITRO ASSAYS TO DETERMINE THE OCULAR TOXICITY OF DETERGENT, OIL, AND ORGANIC SOLVENTS. J Toxicol, Cutan Ocul Toxic 1993;12(1):15-24. (REFS 23)

The cytotoxicity of 39 chemicals (detergents, oils, and organic solvents) was assessed with respect to Chinese-hamster fibroblast (V79) cells, primary rabbit corneal (RC) cells, and normal human epidermal keratinocytes (NHEK). Findings were compared to the Draize test score of 20 (DS20) values from in-vivo Draize eye irritation testing. The relative toxicity of the test chemicals was characterized by the concentration inducing 50% reduction of neutral red uptake compared with in-vitro cytotoxicity (IC50) values of control cell cultures. Results showed that cationic detergents were most toxic to each cell type. A good correlation was noted between DS20

values and IC50 values. Correlation coefficients were as follows: V79 versus DS20, 0.93; RC versus DS20, 0.92; and NHEK versus DS20, 0.90. The authors conclude that the neutral red cytotoxicity assay for V79 cells is helpful for the screening of chemicals irritating to the eyes.

136
Prinsen MK, Koeter HBWM. JUSTIFICATION OF THE ENUCLEATED EYE TEST WITH EYES OF SLAUGHTERHOUSE ANIMALS AS AN ALTERNATIVE TO THE DRAIZE EYE IRRITATION TEST WITH RABBITS. Food and Chemical Toxic 1993;31(1):69-76. (REFS 13)

The use of the enucleated eye test (EET) using eyes of slaughterhouse animals as an alternative to the Draize test for eye irritants was investigated. In the EET, three parameters used to reveal adverse effects of 21 test materials (previously classified by the Commission of the European Communities (CEC)), were corneal thickness, corneal opacity, and fluorescein retention. Species selected as possible eye donor candidates were cow, pig, and chicken. Preliminary availability and suitability tests, dissection procedures for obtaining eyes, and measurement of irritancy in each parameter were described. Results of the preliminary observations showed that chicken eyes offered the best technical possibilities. The comparative study was therefore carried out with enucleated chicken eyes. Mean corneal swelling percentages ranged from -1% (minor shrinkage) to 60%. Corneal opacity scores ranged from 0.0 to 3.1, while mean fluorescein scores ranged from 0.0 to 3.0. Other effects observed after different chemical exposures included loosening of epithelium, pitting, adherence of the test substance to the cornea, and the formation of small vesicles in or on the cornea. On the basis of the mean values for each of the parameters, the test chemicals were assigned to three categories. The chicken EET correctly classified the compounds categorized by the CEC as irritant (R36) or severely irritant (R41). It additionally permitted a third category of slightly irritant compounds to be recognized. The authors conclude that the chicken EET provides a very accurate means of assessing eye irritant potential without using laboratory animals.

137
Kristen U, Hoppe U, Pape W. POLLEN TUBE GROWTH TEST: NEW ALTERNATIVE TO THE DRAIZE EYE IRRITATION ASSAY. J Soc Cosmet Chem 1993;44(May-Jun):153-162. (REFS 27)

To compare the pollen tube growth test to the Draize eye irritation assay, each of 22 tensides were cultivated with pollen, prepared with dye, and measured photometrically;

results were compared with results from Draize eye irritation assays. Results indicated that highly significant rank correlations between the 2 assays were observed. However, in both assays, the most toxic substances were among the group of anionic sulfate/sulfonate tensides, whereas the nonionic tensides showed moderate effects. It was concluded that the pollen tube growth test may be well suited to screen irritation potentials of surfactants.

138
Tsuchiya T, Arai T, Ohhashi J, Imai K, Kojima H, Miyamoto S, Hata H, Ikarashi Y, Toyoda K, Et al. RABBIT EYE IRRITATION CAUSED BY WEARING TOXIC CONTACT LENSES AND THEIR CYTOTOXICITIES: IN VIVO/IN VITRO CORRELATION STUDY USING STANDARD REFERENCE MATERIALS. J Biomed Mater Res 1993; 27(7):885-893.

To clarify the relationship between eye irritancy and cytotoxic potential induced by irritant materials, we made lenses coated with standard reference material (SRMs) prepared from various amounts of zinc diethyldithiocarbamate (ZDEC) and polyurethane (PU). Zinc diethyldithiocarbamate was classified as a mild irritant by the Draize eye irritation test. When ZDEC-SRM coated contact lenses were applied to rabbit eyes, draize scores increased in proportion to both the ZDEC and PU concentrations used for coating. Furthermore, correlation with the cytotoxic potential (gamma = 0.93) was better than with lactate dehydrogenase (LDH) activities of tears from rabbit eyes wearing these coated lenses (gamma = 0.78). In conclusion, in vivo eye irritancy induced by wearing lenses could be estimated quantitatively with the cytotoxic potentials using a colony assay. Furthermore, we could compared different sites of tissue. As a result, the order of sensitivity was eye > muscle skin.

139
Pels E, Nuyts RM, Breebaart AC, Hartmann C. RAPID QUANTITATIVE ASSAYS FOR CORNEAL ENDOTHELIAL CELL VIABILITY IN VITRO. Cornea 1993;12(4):289-94.

The Janus green photometric technique and the Mosmann's colorimetric MTT-assay to quantify corneal endothelial cytotoxicity were compared. Detergents, studied before by in vitro corneal perfusion, proved to be toxic at the 1% level in the Janus green assay with human corneas and at the 0.4% level in the MTT-assay with bovine endothelial cells. These results correlated well with earlier findings in vitro and in vivo. The use of human corneas reduced the ability of the Janus green photometric technique as a fast screening method because

of the heterogeneity and restricted availability of this tissue. With the MTT-assay, large test series could be performed at the same time using cultured cells. Both assays are good tools to screen the cytotoxicity of chemicals and reduce the number of animals needed for endothelial cytotoxicity testing.

140
Tsuchiya T, Arai T, Ohhashi J, Imai K, Kojima H, Miyamoto S, Hata H, Ikarashi Y, Toyoda K, Takahashi M, et al. RABBIT EYE IRRITATION CAUSED BY WEARING TOXIC CONTACT LENSES AND THEIR CYTOTOXICITIES: IN VIVO/IN VITRO CORRELATION STUDY USING STANDARD REFERENCE MATERIALS. J Biomed Mater Res 1993; 27(7):885-93.

To clarify the relationship between eye irritancy and cytotoxic potential induced by irritant materials, we made lenses coated with standard reference materials (SRMs) prepared from various amounts of zinc diethyldithiocarbamate (ZDEC) and polyurethane (PU). Zinc diethyldithiocarbamate was classified as a mild irritant by the Draize eye irritation test. When ZDEC-SRM coated contact lenses were applied to rabbit eyes, Draize scores increased in proportion to both the ZDEC and PU concentrations used for coating. Furthermore, correlation with the cytotoxic potential (gamma = -0.93) was better than with lactate dehydrogenase (LDH) activities of tears from rabbit eyes wearing these coated lenses (gamma = 0.78). In conclusion, in vivo eye irritancy induced by wearing lenses could be estimated quantitatively with the cytotoxic potentials using a colony assay. Furthermore, we could compare different sensitivities caused by the same set of SRMs among three different sites of tissue. As a result, the order of sensitivity was eye > muscle >> skin.

ORGAN CULTURE
141
Ebina M, Hoyt RF Jr, Sorokin SP, McNelly NA. CALCIUM AND IONOPHORE A23187 LOWER CALCITONIN GENE-RELATED PEPTIDE-LIKE IMMUNOREACTIVITY IN ENDOCRINE CELLS OF ORGAN CULTURED FETAL RAT LUNGS. Anat Rec 1993;236(1):226-30.

Small-granule endocrine cells differentiate in airway epithelium of intact and cultured fetal rat lungs. The authors noted that the cells store CGRP in vitro as well as in vivo and used the ionophore A 23187 to test the effects of Ca on peptide secretion in this system. Lungs of 14-day and 15-day fetal rats, organ cultured for 6-9 days, were divided into groups of 5 explants each and incubated for 15 min at 37.degree. in the basic medium contg. 0 mM, 1 mM, or 10 mM

CaCl2, with or without 8 muM A 23187, or 10 mM EGTA. Intracellular CGRP in these explants was quantified by supraoptimal diln. peroxidase immunocytochem. (Springall, D.R; et al., 1988): counts were made of endocrine cells stained with a 1/60,000 diln. of anti-CGRP and repeated on the same sections after restaining with antibody dild. at 1/1000. Results, analyzed by Chi-square test, were expressed as % cells stained with antibody at 1/60,000 vs. those stained at 1/1000. Immunoreactivity for CGRP was reduced by A 23187 in the presence of high extracellular Ca2+ (10 mM), the inference being that these cells secrete peptide hormones in response to Ca2+ influx across the plasma membrane. The organ cultures evidently can be used to assess certain physiol. responses of lung endocrine cells in an accessible, relatively organotypical setting.

142
Egawa M, Hisazumi H, Uchibayashi T, Tanaka M, Sasaki T. COMPARATIVE STUDY OF 3-(4,5-DIMETHYLTHIAZOL-2-YL)-2, 5-DIPHENYLTETRAZOLIUM BROMIDE AND TRITIATED THYMIDINE IN A CHEMOSENSITIVITY TEST USING COLLAGEN GEL MATRIX. Urol Res 1993;21(2):83-8.

An organ culture system using collagen gel matrix (CGM) was used for the detn. of antitumor drug sensitivities. The cell viability of a tumor fragment was measured by the redn. of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a colored formazan product, which allowed for quant. and simple analysis. The sensitivities of KK-47 bladder tumor from nude mice to various anticancer drugs tested corresponded closely to those determined in the original CGM assay system with tritiated thymidine, which has a high clin. correlation. This modified method can be used as a highly reproducible chemosensitivity test in vitro.

143
Yoshino J, Kojima T, Shimizu M, Tomizuka T. CRYOPRESERVATION OF PORCINE BLASTOCYSTS BY VITRIFICATION. Cryobiology 1993; 30(4):413-22.

The objective of this study was to assess the possibility of cryopreservation of porcine expanded and hatched blastocysts by vitrification. The following four types of vitrification solutions were applied in this study: (i) EFT, a mixture of 7.2 M ethylene glycol, 0.003 M ficoll, and 0.3 M trehalose; (ii) DAP213, 2 M dimethyl sulfoxide, 1 M acetamide, and 3 M propylene glycol; (iii) DAP213-T, DAP213 supplemented with 0.3 M trehalose; and (iv) EPT, 4 M ethylene glycol, 3.1 M propylene glycol and 0.3 M trehalose. The embryos collected on

Days 5 to 7 (Day 0 = onset of estrus) were allocated to eight experimental groups according to the types of vitrification solution and the developmental stage. In the toxicity test, the embryos were equilibrated in the respective vitrification solutions either in a single step or in four steps and then transferred to 1 M sucrose in a single step at 22 degrees C without cooling. As a whole, the viabilities of embryos equilibrated in the solutions were lower than those of control embryos. The stepwise equilibration was superior to a single-step equilibration in the in vitro survival of, especially, expanded blastocysts after dilution. No significant difference was observed between the vitrification solutions for the four-step method. In the vitrification test, the embryos were equilibrated in the solutions by the four-step method, loaded into a 0.25-ml plastic straw, and plunged into liquid nitrogen. Although viable embryos were obtained after warming from all of the combinations except the hatched blastocyst-EPT group, viabilities were further reduced by cooling (range of reduction rates: 60 to 100%). The possible cause of low survival after warming is also discussed concerning the cryophysical properties of vitrification solutions.

PULMONARY TOXICITY

144

Chen LC, Fang CP, Qu Q-S, Fine JM, Schlesinger RB. A NOVEL SYSTEM FOR THE IN VITRO EXPOSURE OF PULMONARY CELLS TO ACID SULFATE AEROSOLS. Fundam Appl Toxic 1993;20(2):170-176. (REFS 18)

A system for exposing pulmonary cell cultures to sulfuric-acid aerosols was developed. The system consisted of an aerosol generation and delivery component and a cell exposure component. The former consisted of a Retec nebulizer, preimpactor, and an inertial impactor. The cell exposure subunit consisted of an electric motor driven exposure platform, a speed controller for the motor, and reversing switches. The moving exposure platform was designed to accept two triangular cuvettes, one that contained the cells and another for collecting particles to be used to characterize the physicochemical properties of the aerosol. The cells were cultured in a modified air/liquid interface system designed to simulate liquid aerosol deposition within the airway lumen. The cells were exposed on their apical side and nourished from their basolateral side through a fibronectin enriched permeable membrane. When operating, the system could uniformly deliver sulfuric-acid particles larger than 0.7 micrometer with constant mass concentrations to the target cells. Particles larger than the specified size of interest were removed by the impactor. The system was tested using human

tracheal epithelial cells and aerosols generated from dilute sulfuric-acid solutions. Changes in intracellular pH were used as markers of acid aerosol exposure. The aerosols caused changes in intracellular pH that increased with increasing mass concentration for a given particle size and with decreasing particle size for a given mass concentration. The authors conclude that the in-vitro cell exposure system produces exposed cells that can be used in biological assays.

145
Brain JD, Beck BD. ASSESSMENT OF PARTICULATE TOXICITY. Health Issues Related to Metal and Nonmetallic Mining, W. L. Wagner, W. N. Rom, and J. A. Merchant, Editors; Butterworth Publishers, Boston, Massachusetts, pages 51-61. 1983. (REFS 23)

The assessment of the toxic health effects of particulate matter generated during mining operations was considered. The types of lesions produced on inhalation of particles were inflammation, proteolysis, fibrogenesis stimulation, pulmonary edema, and macrophage phagocytic activity changes. Infections were usually secondary to the toxic effects of the dust and the damage to defense mechanisms. The anatomical, biochemical, and physiological changes induced by particulates were detectable by a variety of tests which were listed. Cellular and enzymatic changes were of particular interest for fibrosis and emphysema evelopment. The in-vitro and tissue culture systems developed for monitoring lung lavage constituents at different times after exposure were discussed. Macrophages played a key role in both defense as well as in pathogenesis. Animal bioassays as models of human chronic lung diseases were described. Short term tests such as the lactate-dehydrogenase isoenzyme test, and the elastase and neutrophil assays for diagnosis of nonneoplastic lung damage were helpful. The need to have better methods for monitoring human exposure to mine dusts was addressed. Since the mine environment contained a variety of toxic materials which had the potential to have synergistic and antagonistic effects, it was not practical to analyze each component separately, and to presume simple additive effects. The authors stress that there is a need for short term bioassays and tests using animal models for the prediction of human risk of lung disease in the mine environment.

146
Chen LC, Fang CP, Qu QS, Fine JM, Schlesinger RB. A NOVEL SYSTEM FOR THE IN VITRO EXPOSURE OF PULMONARY CELLS TO ACID SULFATE AEROSOLS. Fundam Appl Toxicol 1993;20(2):170-176.

While ambient acid aerosols are considered a potential respiratory health hazard, the mechanism by which they induce responses in the lungs is not known. Attempts to ascertain these mechanisms using inhalation exposures are complicated by a number of technical difficulties, chief among which are neutralization of inhaled acids by endogenous ammonia and variations in deposition with inhaled particle size. To control for these variables, a novel in vitro exposure system allowing experimental evaluation of factors which influence biologic responses to acid sulfate particles was developed. The system consists of two subunits, a generation/delivery component and a cell exposure component. Sulfuric acid aerosols are generated by nebulizing dilute acid solutions. Particles larger than a specified size of interest (based upon the specific exposure conditions desired) are removed, and particles at the desired size and mass concentration are uniformly delivered onto a target cell monolayer. The system is capable of delivering acid particles larger than 0.7 mum (mass median diameter), yet at constant particle mass concentrations. This paper describes the design of the exposure system and its performance characteristics and presents initial results of some biological responses obtained using it. In conjunction with inhalation studies, this exposure system may provide additional insights into mechanisms by which acid aerosols adversely affect the respiratory tract and into the physical characteristics of acid particles which modulate toxicity.

147
Wall DA, Pierdomenico D, Wilson G. IN VITRO PULMONARY EPITHELIAL SYSTEM FOR EVALUATING PEPTIDE TRANSPORT. J Controlled Release 1993;24(May 1):227-235. (REFS 34)

An in vitro method to evaluate peptide transport across the respiratory epithelia utilizing Xenopus lung, which resembles mammalian lung morphologically and physiologically, is described. The transport of several model hydrophilic and hydrophobic compounds was linear over 3 h. The relative rates of drug transport correlated well with their relative rates of disappearance from rat lung in vivo. It was concluded that the properties of the Xenopus lung system make it appropriate for use in analyzing the rates and mechanisms of peptide transport across the pulmonary epithelium.

REGULATORY TOXICOLOGY
148
Koeter HB. TEST GUIDELINE DEVELOPMENT AND ANIMAL WELFARE: REGULATORY ACCEPTANCE OF IN VITRO STUDIES. Reprod Toxicol

1993;7(1):117-23. (REFS 11)

Toxicity studies are necessary in order to be able to identify the potential hazards of chemical interference with human reproduction. Until today, most useful contributions to the assessment of possible human reproductive toxicity are considered to be made by animal studies. The OECD Test Guidelines provide the international standards for safety testing and, consequently, have traditionally focused on animal studies. However, the OECD Member countries consider the welfare of laboratory animals also of importance and are of the opinion that animal welfare considerations should significantly influence the work in the OECD Chemicals Programme. A constant effort is being made to discover alternative testing systems and to achieve their regulatory acceptance. However, activities predominately focus on finding alternatives to existing animal studies, rather than developing nonanimal tests that could contribute significantly to the hazard identification process. An approach based on the selection of endpoints essential for hazard identification that would focus on demonstrating similarities with the "real life" target events is considered likely to achieve regulatory acceptance much earlier than an approach based on high correlations between the alternative method and the existing animal study it is supposed to replace, simply because it is never better than the existing method, but at most "almost as reliable."

149
Scott RC, Carmichael NG, Huckle KR, Needham D, Savage T.
METHODS FOR MEASURING DERMAL PENETRATION OF PESTICIDES. Food Chem Toxicol 1993;31(7):523-9. (REFS 16)

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 equivalent to a working day (8 hr). 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 physicochemical properties of the test molecule as well as cost and resource/technical 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 standard defined conditions.

REPRODUCTIVE TOXICITY
150
PROCEEDINGS OF THE INTERNATIONAL WORKSHOP ON IN VITRO METHODS IN REPRODUCTIVE TOXICOLOGY. Ottawa, Canada, 19-20 May 1992. Reprod Toxicol 1993;7(1):1-173.

No abstract.

151
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 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-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 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 sothat 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.

152
Lamb JC 4th, Chapin RE. TESTICULAR AND GERM CELL TOXICITY: IN VITRO APPROACHES. Reprod Toxicol 1993;7(1):17-22. (REFS 25)

Research on testicular toxicology has been advanced significantly by the introduction of in vitro testing

systems. In vivo systems, however, are still essential parts of the risk assessment process, and they are unlikely to be eliminated by in vitro model systems. While in vivo systems are needed to study the integrated male reproductive system, in vitro systems are uniquely suited to investigate specific mechanisms of action in the testis. In vitro systems substantially improve the interpretation and use of in vivo systems. In vitro models can be used alone or in combination with each other to test hypotheses about testicular toxicity. Numerous systems are described in the literature, including Sertoli-germ cell cocultures, Sertoli cell-enriched cultures, germ cell-enriched cultures, Leydig cell cultures, and Leydig- Sertoli cell cocultures. These systems have been used to test relative toxicologic activity of selected chemicals in a class, to investigate the cellular response to certain toxicants, to study the metaboliccapability of cells, and to describe the interaction of adjacent cell types.

153
Webster WS. THE INTERPRETATION OF RESULTS FROM TERATOLOGY AND REPRODUCTIVE TOXICITY TESTS INCLUDING COMMENTS ON THE NEW DRAFT GUIDELINES. Senten Ijo 1992; 32(Suppl):S99-S110.

The preparation of new guidelines for teratology and reproductive toxicity testing presents the opportunity to improve the current guidelines. The existing guidelines were designed to detect hazard and it is only when they are used to determine risk that their limitations are exposed. Risk assessment would be greatly improved if in vivo testing procedures incorporated methodology to compensate for pharmacokinetic and metabolic differences between the test species and humans. This presentation examines results from conventional teratology testing of four drugs which have

subsequently been shown to be human teratogens. These results are compared with data obtained from testing the same compounds using in vitro rat embryo culture where attention is given to the serum concentration of the compound rather than

the dose. The serum concentrations which cause teratogenicity in the in vitro system correlate well with the human plasma levels associated with teratogenicity.

154
Palmer AK. INTRODUCTION TO (PRE)SCREENING METHODS. Reprod

Toxicol 1993;7(1):95-8. (REFS 3)

In summation I start this session with the opinion that in vitro methods cannot be considered as adequate replacements for entire animals at the level of regulatory testing. But, when used to identify mechanisms of action, they can be extremely useful as secondary stage supporting studies. They are of doubtful value for general purpose, broad spectrum screening of single chemical entities or for priority selection of unrelated chemicals. They can be of value for priority selection of homologous series with a known, specific effect on reproduction or development. Such situations are most likely to be present in chemical and drug manufacturing industries where judicious use of in vitro methods in an integrated approach could reduce the number of failures at the later stage of full scale testing. Whether I will need to revise my opinions at the end of this session will depend upon what our speakers have to offer.

STRUCTURE/ACTIVITY TOXICITY

155

Kavlock RJ. STRUCTURE-ACTIVITY RELATIONSHIPS (SARS) OF ENVIRONMENTAL AGENTS IN VIVO AND IN VITRO. Teratology 1992;46(3):10A-11A.

In drug development, SARs are an integral part of the process of finding efficacious and non-toxic analogues. In contrast, SARs are not often a component of hazard identification for environmental agents. This presentation will review efforts to develop SARs for environmental chemicals, with emphasis on comparisons between responses in vivo and in vitro. Relatively large (greater than 10 chemicals) datasets are available only for a few chemical classes: N-alkyl ureas, diphenyl ethers, aliphatic acids, dioxins, glycol ethers, triazole fungicides, and substituted phenols. In the first three examples, formal SARs were not sought although active structural attributes were sometimes identified. For these, in vitro data is available only for the aliphatic acids. With a few notable exceptions, data from whole embryo culture do not demonstrate the structural requirements observed in vivo. For dioxins, the SAR is based upon in vivo data and relates the developmental toxicity to the ability to bind to and activate the Ah receptor. For the glycol ethers, the developmental toxicity is dependent on generation of a particular metabolite. This requirement is supported by whole embryo culture data. The triazoles have been studied in micromass culture, with effects dependent on log P. Limited in vivo studies support the in vitro findings. The most extensive comparison of in vitro versus in vivo SAR for environmental chemicals has been with substituted phenols. In rat whole embryo culture, log P and molar refractivity largely explained the ability of the congeners to induce growth retardation and dysmorphogenesis.

No similar SAR was found when the congeners were administered to rats at the corresponding stage of pregnancy. The strengths and weaknesses of these various approaches will be discussed in light of hazard identification and in vitro test validation.

TEROTOGENICITY
156
Spielmann H. RISK EVALUATION IN TERATOLOGY - ANIMAL MODELS WITH HUMAN RELEVANCE. Reprod Toxicol 1992;6(2):187-8.

Many investigators have recommended the use of specific species of laboratory animals to assess risk to the human. There is no a priori basis for selecting a certain species as a suitable model for predicting the hazard to humans. Testing in animals shows that with respect to the few known human teratogens no one species of laboratory animals has been clearly satisfactory. It is generally assumed that for realistic testing the maternal-placental-embryonic relationship is essential. Predictive developmental toxicity of chemicals should be assessed in those laboratory animal species that are most likely to reflect toxicity in man. For testing in developmental toxicity, laboratory animal studies have to be evaluated for their sensitivity (ability to detect a true positive response in humans) and specificity (ability to detect a true negative response in humans). Of 38 compounds having demonstrated or suspected teratogenic activity in humans, all tested except one (tobramycin, which causes otologic deficits in humans) were positive in at least one animal species. In addition, more than 80 were positive in

multiple species. The overall findings indicate that conventional animal species (mouse, rat, rabbit) have high sensitivity for human teratogens. There is no assurance, on the other hand, that negative results by testing chemicals in these species can be used to predict that an agent will lack teratogenic effects in humans. Tests required by regulatory agencies are often referred to as "screening" or "primary stage testing". Such studies are only part of a composite of investigations that are considered to be sufficient to allow judgement as to whether humans can be exposed or whether further animal studies -- commonly referred to as "secondary stage testing" -- need to be performed. Generally, "secondary stage testing" is aimed at more specific aspects of prenatal development to further clarify the effects observed in the screening tests. Such studies have to be designed for the problem to be investigated and may include in vitro testing. Primary and secondary stage testing are recommended by the EEC.

157
Waalkens-Berendsen DH, Smits-van Prooije AE, Koeter HB, Leeman WR, Dijkstra A. POTENTIAL TERATOGENIC EFFECTS OF SOME GLYCOALKALOIDS. Teratology 1992;46(3):31A.

The literature on the teratogenic properties of glycoalkaloids is conflicting. At present, the teratogenic effects of the glycoalkaloids alpha-solanine, alpha-chaconine and tomatine are being examined in our Institute, using two different test systems: an in vivo test system in pregnant Wistar rats and an in vitro test system called the Micromass assay. Pregnant females were treated once by gavage, on day 9 of gestation, with 50 mg/kg alpha-solanine, 50 mg/kg alpha-chaconine or 250 mg/kg tomatine dissolved in propylene glycol. Retinol palmitate 75,000 IU per animal, dosed on day 8 and 9 of gestation, was used as positive control. During pregnancy body weight, body weight gain and food consumption were recorded. The females were killed on day 21 of gestation. At Caesarian section the number of corpora lutea, implantation, early and late resorptions, live and dead foetuses, foetal weight and length and foetuses with external abnormalities were recorded. The foetuses were screened for either skeletal or visceral abnormalities. In the in vivo studies the females treated with 250 mg tomatine/kg gained less weight and showed an increased number of early resorptions as compared with the vehicle-treated controls. Conspicuous effects of retinol palmitate treated foetuses found in the in vivo study were exencephaly, micrognatia and skeletal abnormalities. In the foetuses of the females treated with glycoalkaloids no such conspicuous effects were observed. Tomatine as tested in the Micromass assay appeared to be a potential teratogen as indicated by the IC50 differentiation of 8.4 ug/mL and 0.9 ug/mL and the IC50 survival/IC50 differentiation of 3.8 and 10 for limb bud cells (chondrocytes) and mid brain cells (neurons), respectively.

158
Amacher DE, Stadler J, Schomaker SJ, Verseil C. POSSIBLE DEVELOPMENTAL EFFECTS OF SOME ARYL TRIAZINE ANTICOCCIDIAL AGENTS IN RAT LIMB BUD MICROMASS CULTURES AND IN RAT EMBRYOCULTURE. Teratology 1992;46(3):19A.

When cultured at high density, limb bud mesenchyme cells isolated from 13 day rat embryos proliferate and differentiate into chondrocytes providing an in vitro model for early skeletal development. In this system, suspected teratogens diminish cartilage proteoglycan synthesis thus inhibiting cell differentiation, an effect predictive of teratogenicity in

vivo (Flint & Orton, Toxicol. & Appl. Pharmacol. 76:383, 1984). In this study the amount of drug required to cause a 50% inhibition of alcian blue uptake (PG50) by cartilage proteoglycans in spot cultures of limb bud cells was used to assess teratogenic potential in vitro following 48 hour exposure to each of 4 anticoccidials, 3 metabolites, and, for comparison, 6-azauracil. Following drug removal, cultures were incubated another 96 hours, then cells were fixed and stained with 0.5% alcian blue. Bound dye was extracted and quantitated. In parallel cultures, cell viability was measured by neutral red uptake and protein content was assayed by the BCA method. Test concentrations were usually 5-250 ug/mL. PG50 values less than 50 ug/mL were obtained for CP-25,415 and its metabolite CP-25,641; CP-30,542 and its metabolites CP-37,537 and CP-32,107; and CP-25,722 suggesting potential teratogenicity as described by Flint (Fd. Chem. Toxic. 24:627, 1986). PG50 values for CP-21,745 and 6-azauracil were greater than 250 ug/mL suggesting no teratogenic potential. Both CP-32,107 and CP-25,722 were considerably less cytotoxic than the other 4 in vitro proteoglycan synthesis inhibitors, suggesting greater potency as developmental toxicants. When 3 of these compounds (CP-25,722, CP-25,415 and CP-30,542) were tested in a 48-hour rat embryoculture model, dysmorphogenicity was evidenced for all of them. CP-25,722 induced developmental defects at 30 ug/mL, a dose which was not embryotoxic. CP-25,415 and CP-30,542 produced these defects only at higher concentrations, in the presence or absence of embryotoxicity, respectively. The potency of CP-25,722 as a developmental toxicant has been confirmed in vivo where it was teratogenic in rats at a dose level which was devoid of any other embryo- or fetotoxicity and in the absence of any maternal toxicity. The absence of teratogenic potential of CP-21,745 in the limb bud micromass culture has been also confirmed in an in vivo study in rats.

159
Nito S, Ariyuki F, Nakayama Y. A NEW IN VITRO SCREENING METHOD FOR TERATOGENS USING HUMAN EMBRYONIC PALATAL MESENCHYMAL CELLS. Senten Ijo 1991;31(4):329-36.

In order to establish an in vitro screening assay system for cleft palate- inducing teratogens, we tested 31 teratogenic and 10 nonteratogenic compounds using human embryonic cultured cells. We examined whether cleft palate- inducing ability can be detected by differential growth inhibition between human embryonic palatal mesenchymal (HEPM) cells and human embryonic fibroblasts (MRC-5). Thirty one compounds with proven cleft palate-inductive effects in vivo preferentially inhibited the proliferation of HEPM cells. The average of the relative resistant rates (rate of IC50 value for HEPM cells to

MRC-5 cells) of teratogens was 0.53. In contrast, almost all nonteratogens identically inhibited the proliferation of both cell lines and the average of the relative resistant rates was 1.01. These results show that teratogens which induce cleft palate in vivo preferentially inhibit the proliferation of embryonic palatal mesenchymal cells. The data indicated that in vitro screening using HEPM and MRC-5 cells is useful for detecting the cleft palate-inducing ability of chemicals.

160
Schumacher GH. FINDINGS AND POSSIBILITIES IN TERATOLOGY. Senten Ijo 1992;32(1):1-13.

Reports of malformations are already documented in the writings of Assyrian and Babylonian astrologists dating back 1800 before our chronology. Historically, a significant source of informations are pamphlets. These publications started in Germany in the 15th century to disseminate news. For most congenital anomalies the causes are unknown. The majority of developmental defects have a multifactorial etiology and are induced interactions of environmental and genetic factors. In order to examine the effect of noxae the development of mammals is subdivided into several segments. On the base of guidelines which were issued by the Food and Drug Administration (FDA) in 1966 the principles of in-vivo teratogenicity testing procedures are discussed. As a rule reproduction toxicological investigations are carried out on mice, rats and rabbits. Special attention is to be paid on dose limits, mode of application, toxicokinetics of the test agents, morphological criteria and specific disorders of developmental rhythm. Stimulating factors for the use of in-vitro tests are the increasing number of chemical substances. Advantages and disadvantages of in-vitro technique are discussed. Some of the actual in-vitro models are presented, such as monolayer culture, organoid culture, organ cultures, "whole embryo" culture. It is concluded that in-vivo experiments cannot be replaced by in-vitro systems, but its usefulness in teratological research has been proven.

161
Spezia F, Lozes P, Fournex R, Vannier B. QUANTITATIVE STRUCTURE-TERATOGENICITY RELATIONSHIP AND CORRELATION BETWEEN THE IN VIVO AND IN VITRO TERATOGENIC ACTIVITY OF PHENOTHIAZINE DERIVATIVES. Teratology 1992;46(3):28A.

Phenothiazine derivatives are used in therapeutics for their antihistaminic and antipsychotic properties. The induction of fetal malformations in animal has been demonstrated with several of these drugs and it has been postulated that the

teratogenic activity could be related to the chemical structure, in particular to the presence of a 3-carbon aliphatic side chain. The purpose of this study was to attempt to establish a quantitative structure-teratogenic activity relationship and a correlation between the in vivo and in vitro results. Firstly, more than forty phenothiazines were tested in vitro in the rat limb bud cell assay. Concentrations inducing 50% inhibition of proliferation or differentiation and provoking a minimal cytotoxic effect were measured. Moreover, lipophilic (log P), steric (MR) and electronic (sigma P) constants were calculated. Secondly, the in vivo effects of these phenothiazines were investigated in a limited embryotoxicity test in mouse. Pregnant females were treated with the maximum tolerated dose on gestation days 6-10. The embryotoxicity was assessed by the survival rate and development of newborn within the three days after birth. According to the calculated physico-chemical parameters, the teratogenic potency of some phenothiazines seems to be related not only to a 3-carbon side chain but also to a pyrazine structure. Furthermore, an in vitro - in vivo correlation was observed for most of the compounds.

162
Newman LM, Johnson EM, Haghoost NR. A TOXIC AND TERATOGENIC POTENTIAL RANKING OF SODIUM ARSENATE SODIUM ARSENITE AND CACODYLIC ACID BY THE APPLICATION OF THE IN-VITRO HYDRA ASSAY. Thirty-Third Annaul Meeting of the Teratology Society, Tucson, Arizona, USA, June 28-July 1, 1993. Teratology 1993; 47(5):430-431.

No abstract.

TISSUE CULTURE
163
Lucas-Clerc C, Massart C, Campion JP, Launois B, Nicol M. LONG-TERM CULTURE OF HUMAN PANCREATIC ISLETS IN AN EXTRACELLULAR MATRIX: MORPHOLOGICAL AND METABOLIC EFFECTS. Mol Cell Endocrinol 1993;94(1):9-20.

In this experiment, various conditions for embedding cultures of human pancreatic islets in type I collagen gel were studied in an attempt to maintain the highly differentiated functions of islet cells and particularly insulin secretion over a long period of time. The islets isolated by a collagenase digestion

technique were plated either on or within the collagen gel and refed with either Eagle's min. essential medium (5.5 mM D-glucose) or RPMI 1640 medium (11 mM D-glucose) supplemented

with 10% FCS and antibiotics. The comparison between the 2

culture media showed that embedded islets cultured in RPMI had a higher basal insulin secretion rate, survived longer than their MEM counterparts, but exhibited impaired response to an acute glucose test contrasting thus with islets cultured in MEM. The secretory behavior of islets was also related to the different morphol. modifications occurring during culture. Islets directly embedded within the collagen gel more or less maintained their spherical structure and highest secretory capacities. When overlaid with a second layer of collagen, well established monolayers of human islet cells grown on collagen underwent a gradual and complete reorganization into a 3-dimensional islet-like structure with a striking

reinforcement of their secretory activity. Both cultures were able to survive more than 8 wk, thus proving the usefulness of such a new model for long-term culture. In contrast, std. cultures on culture treated plastic dishes on which islets cells rapidly established wide monolayers, exhibited a rapid and definitive decline in insulin secretion with a survival not exceeding 14 days. In the light of these different culture conditions, possible mechanisms responsible for disturbance of hormonal release and their implications for in-vitro study of isolated islets functions are discussed. This work is an example of the permissive effects of collagen matrixes on the establishment or maintenance of tissue-like structures in vitro, sugesting the definition of a new model for the study of human pancreatic islets in long-term culture.

164
Gonzalez A, Oberley TD, Schultz JL, Ostrom J, Li JJ. IN VITRO CHARACTERIZATION OF ESTROGEN INDUCED SYRIAN HAMSTER RENAL TUMORS: COMPARISON WITH AN IMMORTALIZED CELL LINE DERIVED FROM DIETHYLSTILBESTROL-TREATED ADULT HAMSTER KIDNEY. In Vitro Cell Dev Biol Anim 1993;29A(7):562-73.

Primary diethylstilbestrol-induced kidney tumors from Syrian hamsters were grown in vitro and maintained in culture for 6 mo. Combined immunohistochemical studies using antibodies to intermediate filaments and ultrastructural studies of tumor cells in culture exhibited characteristics similar to tumor cells in vivo. Furthermore, the cells manifested transformed properties in culture; they grew both as multilayered colonies attached to the tissue culture substrate and as floating multicellular colonies (spheroids). When cultured cells were injected into diethylstilbestrol-treated recipient hamsters, tumors developed at the injection sites. In contrast, renal tubules or whole kidney cortex from control hamsters cultured in the same medium underwent only short-term growth, with

senescence developing after approximately 1 mo. However, cell cultures of kidney cortex from animals treated in vivo for 5 months with diethylstilbestrol formed a cell line. This diethylstilbestrol-induced cell line has been maintained in culture for 1.5 yr and has the following characteristics: a) it is anchorage-dependent, b) it is negative in in vivo tumorigenicity tests, and c) cultured cells are histochemically and ultrastructurally similar to cultured tumor cells. This culture system should prove to be of use in studying hormonal carcinogenesis in vitro.

TOXICOLOGY (GENERAL)

165
Calleja MC, Persoone G. THE INFLUENCE OF SOLVENTS ON THE ACUTE TOXICITY OF SOME LIPOPHILIC CHEMICALS TO AQUATIC INVERTEBRATES. Chemosphere 1993;26(11):2007-22.

In the present study, water-insol. chems. such as diazepam, digoxin, and malathion have been tested either in pure form or in combination with DMSO, ethanol, methanol or acetone, for their acute toxicity to several aquatic invertebrates commonly used in ecotoxicol. The three former compounds are included in the first 20 "priority chems." of the Multicentre Evaluation of In vitro Cytotoxicity (MEIC) program. The results indicate that the use of solvent carrier gave differences in the acute effect of the three chems. to 6 test species: the crustaceans Artemia salina, Streptocephalus proboscideus, and Daphnia magna, the rotifers Brachionus plicatilis and Brachionus calyciflorus, and the bacterium Photobacterium phosphoreum. Consequently, the toxicity resulting from the combination of test substance and solvent should be evaluated carefully, and the magnitude of interaction taken into consideration when comparing toxicity data from various sources.

166
Terse PS, Madhyastha MS, Zurovac O, Stringfellow D, Marquardt RR, Kemppainen BW. COMPARISON OF IN VITRO AND IN VIVO BIOLOGICAL ACTIVITY OF MYCOTOXINS. Toxicon 1993;31(7):913-19.

In vitro assays developed to screen the cytotoxic activity of chems. in murine (NIH/3T3) and bovine (BE 12-6) embryonic cells were used to det. the concns. of mycotoxins which caused 50% lethality (LC50). Embryonic cells were seeded in 96 well plates, cultured for 72 h with dilns. of each individual and combinations of mycotoxins, and stained and counted. Verrucarin A and roridin A had the strongest cytotoxic activity, and ergotamine tartrate was least toxic. Furthermore, results correlated with published values of in vivo activity, indicating this assay can be used

for acute toxicity screening of compds.

TUMOR TOXICITY
167
Hanauske AR, Ross M, Degen D, Hilsenbeck SG, Von Hoff DD. IN VITRO ACTIVITY OF THE BENZOTRIAZINE DIOXIDE SR 4233 AGAINST HUMAN TUMOUR COLONY-FORMING UNITS. Eur J Cancer 1993; 9A(3):423-5.

SR 4233 (3-amino-1,2,4-benzotriazine 1,4-dioxide) is a novel bioreductive agent selectively toxic to hypoxic cells. It is active as a radiation sensitiser in vitro. Using a human tumour cloning system we have studied the effects of SR 4233 against freshly explanted human tumour specimens under hypoxic and non-hypoxic culture conditions. For hypoxic conditions, final concentrations of SR 4233 of 10.0-500 mumol/l were used in short-term (1 h) exposure experiments. Final concentrations in non-hypoxic experiments ranged from 10 to 1350 mumol/l. 25 tumour specimens were tested under each culture condition. Of those, 14 (56%) were evaluable. The most common tumour types recruited included ovarian, non-small cell lung, and breast cancer. A moderate concentration-dependent increase in the frequency of inhibited tumour specimens under non-hypoxic conditions was observed with zero out of 10 sensitive specimens at 10 mumol/l as compared with five out of 14 (36%) sensitive specimens at 500 mumol/l ($P < 0.02$). However, when

hypoxic conditions were used SR 4233 had a profound antitumour activity, (two out of 14 specimens sensitive at 10 mumol/l compared with 10 out of 10 specimens sensitive at 500 mumol/l, $P < 0.00005$). We conclude that SR 4233 is active against tumour colony-forming units in vitro and that its antitumour activity is greatly increased against hypoxic tumour cells.

VALIDATION TESTS
168
Li J, Suzuki Y, Shimizu H, Fukumoto M, Okonogi H, Nagashima T, Ishikawa T. IN VITRO MICRONUCLEUS ASSAY OF 30 CHEMICALS IN CHL CELLS. Jikeikai Med J 1993;40(1):69-83.

A rapid and simple in vitro micronucleus assay in Chinese hamster lung (CHL) cells was performed in the present study. In a preliminary validation of this test system, the authors have investigated 30 chemicals including both carcinogens and non-carcinogens in order to evaluate the sensitivity of this assay in screening of environmental mutagens and carcinogens. Twenty-eight out of 30 chemicals were positive in this assay. Correlations between the formation of micronuclei and induction of chromosomal aberrations in CHL cells were

relatively good. Nine out of 10 antitumor agents yielded pos. results, while in the Ames test 4 were positive results. This assay appears to be relatively highly sensitive and useful in screening chems. of carcinogenic potential. Some problems concerning the mechanism of micronucleus formation are also discussed.

169
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.

170
Kucera P, Honegger P, Zijlstra J, Schmid B. THREE IN VITRO TOXICITY/TERATOGENICITY TEST SYSTEMS VALIDATED BY USING TWELVE IDENTICAL CODED COMPOUNDS. Experientia 1992;48(Abstr):A33.

Toxic and teratogenic effects of 6 pairs of coded chemicals were assessed in 1) cultures of whole chick embryos 2) cultures of whole rat embryos and 3) aggregating brain cell cultures. (For methods, Experientia 44:vol 10, 1988). Dose-dependent general toxic effects were obtained in all 3 systems. Similar types of malformations were induced in both chick and rat embryos. In the brain cell cultures, neuron- and glia-specific effects were distinguished. The compounds could be classified with respect to their teratogenicity/toxicity as follows: 1) less than 10(-6)M: retinoids (Ro 13-6307, Ro

1-5488), 6-aminonicotinamide, ketoconazole; 2) 10(-6) to 10(-3)M: sulfadiazine, 4-hydroxy-pyridine, sulfanilamide, theophylline, caffeine, metronidazole, methoxyacetic acid; 3) greater than 10(-3)M: methoxyethanol. The results are comparable to data available from in vivo experiments.

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Li J, Suzuki Y, Shimizu H, Fukumoto M, Okonogi H, Nagashima T, Ishikawa T. IN VITRO MICRONUCLEUS ASSAY OF 30 CHEMICALS IN CHL CELLS. Jikeikia Med J 1993;40(1):69-83.

A rapid and simple in vitro micronucleus assay in Chinese hamster lung (CHL) cells was performed in the present study. In a preliminary validation of this test system, we have investigated 30 chemicals including both carcinogens and non-carcinogens in order to evaluate the sensitivity of this assay in screening of environmental mutagens and carcinogens. Twenty-eight out of 30 chemicals were positive in this assay. Correlations between the formation of micronuclei and induction of chromosomal aberrations in CHL cells were relatively good. Nine out of 10 antitumor agents yielded positive results while in the Ames test 4 were positive results. This assay appears to be relatively highly sensitive and useful in screening chemicals of carcinogenic potential. Some problems concerning the mechanism of micronucleus formation are also discussed.

XYZ/MISCELLANEOUS
172
Mauel J, Pham TV, Kreis B, Corradin-Betz S, Bauer J. EVALUATION OF ASSAY PROCEDURES MEASURING MACROPHAGE STIMULATION BY IMMUNOMODULATORS IN VITRO. Dev Biol Stand 1992; 77(Standardization of the Immunopharmacology of Natural and Synthetic Immunomodulators):71-7.

Several assay procedures for measuring the stimulatory effect on macrophages of bacterial immunomodulators (OM-85, OM-89, OM-163) were evaluated with regard to their complexity, speed, and general convenience. Murine bone marrow-derived or peritoneal exudate macrophages were exposed to the immunomodulators in vitro, then tested for metabolic stimulation (glucose oxidn. through the hexose monophosphate shunt pathway, synthesis of type E prostaglandins, release of superoxide, and production of L-arginine-derived nitrogen oxidn. products), as well as for the enhancement of functional activities (prodn. of tumor necrosis factor-alpha, extracellular cytolysis of P815 target cells, and intracellular parasite destruction). All the tests provide adequate measurements of the macrophage response to the immunomodulators, with significant effects detectable using the compounds in the ng/mL to mug/mL range. Concomitant incubation

with crude macrophage activating factor or with recombinant murine interferon-gamma (IFN-gamma) dramatically increased the macrophage sensitivity to the immunomodulators, and was an abs. requirement for induction of macrophage cytotoxic activities by the bacterial exts. The measurement of nitrite prodn. by macrophages exposed to the immunomodulators with or without treatment with 10 U/mL of IFN-gamma was a highly convenient procedure which correlated well with functional assays.

172
Gallagher J, George M, Kohan M, Thompson C, Shank T, Lewtas J. DETECTION AND COMPARISON OF DNA ADDUCTS AFTER IN VITRO AND IN VIVO DIESEL EMISSION EXPOSURES. Environ Health Perspect 1993;99(225-8).

The authors have compared diesel-modified DNA adduct patterns in various in vitro and in vivo rodent model systems and compared them to DNA reactive oxidative and reductive metabolites of 1-nitropyrene. The formation of nitrated polycyclic arom. hydrocarbon (nitrated PAH)DNA adducts, derived from the metab. of diesel ext. constituents, was enhanced relative to the PAH-derived DNA adducts via xanthine oxidase-catalyzed nitroredn. These adducts were detectable only by the butanol extn. version of the postlabeling anal. Five major DNA adducts were detected in human lymphocytes treated in vitro with diesel ext. A major adduct detected in human lymphocytes treated in vitro with diesel ext. co-migrated with a major adduct detected in lymphocyte DNA treated with benzol[a]pyrene (BaP) alone. Other adducts that co-migrated with the major BaP-derived adducts were detected in skin and lung DNA isolated from rodents topically treated with (50 mg) diesel ext. and the major adduct detected in calf thymus DNA treated with rat liver S9 and diesel particle ext. Postlabeling of lung DNA isolated from rodents exposed via lung inhalation for 24 mo to diesel combustion emissions resulted in the formation of a major nuclease-P1-sensitive DNA adduct that did not co-migrate with the major BaP-diol epoxide adduct. Based on its sensitivity to nuclease-P1, this adduct may be an N-substituted aryladduct. Marker adducts detected in the various test systems presented here will assist in characterizing nuclease-P1-sensitive nitrated PAH adducts in humans.

173
Hong Y, Winkler C, Brem G, Schartl M. DEVELOPMENT OF A HEAVY METAL-INDUCIBLE FISH-SPECIFIC EXPRESSION VECTOR FOR GENE TRANSFER IN VITRO AND IN VIVO. Aquaculture 1993; 111(1-4):215-26.

The promoter of the rainbow trout metallothionein B gene (tMTb)

was isolated from genomic DNA by the polymerase chain reaction (PCR), fused to the bacterial chloramphenicol acetyltransferase (CAT) gene in an expression vector, and functionally analyzed in 1 human cell line and 4 fish cell lines. This promoter exhibited an extremely low basal expression in all cell lines and was Zn- and Cd-inducible, except in the fish melanoma cell line where the promoter was completely inactive. The metal-induced expression patterns were cell line-specific. In general the fish promoter was more species- and cell type-specific than its human counterpart. In a transient assay, it was functional in developing embryos of the medaka (Oryzias latipes). These properties make this promoter suitable for inducible, tissue-specific expression of transgenes and for in vivo studies of gene function and regulation.

174
Szulczewski DH, Meyer U, Moeller K, Stratmann U, Doty SB, Jones DB. CHARACTERIZATION OF BOVINE OSTEOCLASTS ON AN IONOMERIC CEMENT IN VITRO. Cells Mater 1993;3(1):83-92.

Primary bovine osteoclasts were obtained by an outgrowth method from bovine periosteum and cultured for 7 days on an ionomeric cement for biomaterial testing. Osteoclasts cultured on slices of bovine bone and on glass microscope cover-slides served as a control. The cells were characterized as osteoclasts by a no. of tests. Osteoclasts showed pos. staining for tartrate resistant acid phosphatase and reactivity with the antibodies 13C2 and 23C6, which react with the alpha-chain of the vitronectin receptor. Addition of salmon calcitonin to the culture medium led to sudden cessation of lamellipodial activity. The cells resorbed bone by making pits. In mixed cultures with osteoblasts, the morphol. of the osteoclasts on the smooth ionomeric cement surface was comparable to the one on glass cover-slides, revealing broad cytoplasmatic extensions on the material. Acridine orange staining demonstrated viability of cells until the end of the culture period and increased acidification after parathyroid hormone (PTH) stimulation. SEM did not reveal erosion of the material by osteoclasts. No signs of aluminum toxicity on osteoclasts could be detected during the 7 day culture period, although an increased uptake of aluminum into the cell was demonstrated.

175
Sakuma K, Ohtani H, Kanoh M, Yamamoto K. BIOCHEMICAL STUDY ON THE COSMETIC RAW MATERIALS: I. TYROSINASE INHIBITORY ACTION. Jpn J Toxicol Environ Health 1993;39(3): 226-229.

Tyrosinase inhibitory action (TIA) of cosmetic raw materials containing cosmetic whitening ingredients was measured as an in

vitro screening procedure. Among them, L-ascorbic acid phosphate magnesium slat (VC-PMg) had no TIA. The decomposition of VC-PMg was also examined by acid-phosphatase (Acp), a skin enzyme. Consequently, VC-PMg was hydrolyzed to L-ascorbic acid by pseudo first order reaction. The reaction solution containing L-ascorbic acid had TIA. The whitening effects of several cosmetic raw materials could be compared by the in vitro TIA test.

176
Furst A, Chien Y, Chien PK. WORMS AS A SUBSTITUTE FOR RODENTS IN TOXICOLOGY: ACUTE TOXICITY OF THREE NICKEL COMPOUNDS. Toxic Meth 1993;3(1):19-23.

In a continuing investigation of the use of the common earthworm (Lumbricus terrestris) as test subjects for metal toxicity, three nickel salts were evaluated. Saline solutions of either the chloride, the sulfate or the acetate were injected into the coelom of the worm. Care was taken not to enter the gastrointestinal tract. The acute toxicities were determined by graphical methods using probit paper. The most consistent results were found when lethality was measures at 48 hours rather than at 24 hours. The 48-hour LD50 values for the different salts of nickel are as follows: chloride, 52 mg/kg; sulfate, 54 mg/kg; and acetate, 69 mg/kg. The rations of toxicity of nickel acetate to the chloride and sulfate salts in the worms are similar to those ratios noted in mice. The European Economic Commission (EEC) now suggests using earthworms for toxicity testing for legislation purposes.

177
Chen T, Furst A, Chien P. THE EFFECTS OF CADMIUM AND IRON ON CATALASE ACTIVITIES IN TUBIFEX. J Amer Coll Toxic 1994; 13(2):112-120.

The effects of 1 M Cd(II), alone and in combination with several concentrations of Fe(II), were investigated in Tubifex tubifex (a fresh water worm) by the determination of catalase activities in vivo at 6, 12, 24, and 48 hours. At 6 hours postexposure the catalase activities in all cadmium-exposed groups dropped below control values. AT 12 and 24 hours the values rose; at 48 hours the catalase activities exceeded control values. The combination of 1 M Cd (II) plus 100 M Fe(II) exhibited the least inhibition of catalase activity at 6 hours and the most increased activity at 48 hours. By comparison, the in vitro results of incubating bovine liver catalase for 6 hours with different concentrations of Cd(II) and Fe(II) showed that Cd alone at 10 M had no inhibitory effect on catalase activity and that 100 M Fe(II) on Cd(II)

LC50 values were also studied. Adding 100 M Fe(II) or pretreating for 48 hours with a mixture of 1 M Cd(II) and 100 M Fe(II) decreased the toxicity of Cd(II) by almost 70%. These studies indicate (a) that a mixture of Cd(II) and Fe(II) will increase catalase activities in vivo after a 2-day exposure and (b) that certain concentrations of Fe(II) can protect the worm against Cd(II)toxicity. One suggested mechanism is that Fe(II) can inhibit Cd uptake and transfer. An enhanced catalase activity appears to protect the worms against the toxic effects of Cd(II).