1992 No. 2
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

Prepared By:

Toxicology and Environmental Health Information Program
Specialized Information Services
National Library of Medicine
National Institutes of Health
Bethesda, MD USA

Vera W. Hudson, M.S.
Project Coordinator and Scientific Editor
National Library of Medicine

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.

National Library of Medicine, NIH
Specialized Information Services
Office of Hazardous Substance Information
2 Democracy Plaza, Suite 510
6707 Democracy Blvd., MSC 5467
Bethesda, MD 20892-5467 USA
Telephone: (301) 496-1131
FAX: (301) 480-3537

Email: Vera_Hudson@OCCSHOST.NLM.NIH.GOV

Suggestions and comments are welcome.

BRAIN

1

Dehouck MP, Jolliet-Riant P, Bree F, Fruchart JC, Cecchelli R, Tillement JP. DRUG TRANSFER ACROSS THE BLOOD-BRAIN BARRIER: CORRELATION BETWEEN IN VITRO AND IN VIVO MODELS. J Neurochem 1992;58(5):1790-7.

To assess the drug transport across the blood-brain barrier (BBB), the authors compared the maximal brain extn. values at time O [E(0) values] obtained using either in vitro or in vivo methods. The in vitro BBB model consisted of a co-culture of brain capillary endothelial cells growing on one side of a filter and astrocytes on the other. The in vivo model used intracarotid injection in anesthetized rats. Eleven compounds were tested. They were selected because they exhibit quantitatively different brain extn. rates. The in vivo and in vitro E(0) values showed strong correlation as indicated by the Spearman's correlation coeff. ($r = 0.88$, $p < 0.01$). The relative ease with which such co-cultures can be produced in large quantities could facilitate the screening of new centrally acting drugs.

CARCINOGENESIS

2

Kuper A, Benford DJ. UNSCHEDULED DNA SYNTHESIS IN TRACHEAL EPITHELIAL CELL CULTURES. Toxicol in Vitro 1991;5(5-6):511-13.

An in vitro method has been established for the isolation and culture of tracheal epithelial cells for the evaluation of chemical induced genotoxicity using an unscheduled DNA synthesis assay. Cell cultures were derived from the Wistar albino rat and the golden Syrian hamster. Epithelial cells were isolated with protease type XIV for 16 hours and allowed to attach for 24 hours on collagen-coated cover slips in multi-well plates. Cells were exposed to carcinogens for 24 hours. The test results may indicate a suitability of this culture system for the evaluation of airborne carcinogens.

3

Tiedink H GM, De Haan L HJ, Jongen W MF, Koeman JH. IN VITRO TESTING AND THE CARCINOGENIC POTENTIAL OF SEVERAL NITROSATED INDOLE COMPOUNDS. Cell Biol Toxicol 1991;7(4):371-386.

4-chloro-methoxyindole is a naturally occurring compound in Vicia faba which can easily react with nitrite to form a N-nitroso compound. In this in vitro study, the potential genotoxic effects of nitrosated 4-chloro-6-methoxyindole and its structural analogue 4-chloroindole were evaluated for the first time by using both Salmonella and Chinese hamster V79 cells. The inhibition of gap junctional intercellular communication in V79 cells by these compounds was also determined; this is a validated parameter for tumor-promoting activity. Assays were also performed with nitrosated indole-3-acetonitrile, a naturally occurring compound in brassicas. Both nitrosated chloroindoles were highly mutagenic to Salmonella typhimurium TA100 without the need of exogenous metabolic activation and were potent inducers of sister chromatid exchanges. Results indicate that nitrosated chloroindoles and nitrosated indole-3-acetonitrile should be considered as mutagens and agents with the potential to promote tumors.

4
Gabrielson EW, Lechner JF, Gerwin BI, Harris CC. CULTURED HUMAN MESOTHELIAL CELLS ARE SELECTIVELY SENSITIVE TO CELL KILLING BY ASBESTOS AND RELATED FIBERS: A POTENTIAL IN VITRO ASSAY FOR CARCINOGENICITY. NATO ASI Ser., Ser. A 1991;223(Mech. Fibre Carcinog.):505-11.

Cell killing of cultured normal human mesothelial cells by amosite, chrysotile, or crocidolite fibers occurs at levels of exposure approximately 50 fold less those required to produce cell killing cultured human lung fibroblasts. This differential cytotoxic effect is also observed for erionite, a fibrous zeolite (which has been linked to mesothelioma in Turkey), and Code 100 glass fiber found to cause mesothelioma in laboratory animals. Human mesothelial cells, a selective in vivo target to the carcinogenic effects of asbestiform fibers, are also a selective in vitro target of the cytotoxic effects of these fibers. Differential cytotoxicity for cultured mesothelial cells and fibroblasts may be useful for predicting the potential of new man-made fibers to cause mesothelioma.

5
Ladiges WC. ALTERNATIVES TO THE USE OF CONVENTIONAL RESEARCH ANIMALS IN NEOPLASIA RESEARCH. J Am Vet Med Assoc 1992 Mar 1;200(5):674-6. (11 REFS)

No abstract.

6
Taylor CW, Lui R, Fanta P, Salmon SE. EFFECTS OF SURAMIN ON IN VITRO GROWTH OF FRESH HUMAN TUMORS. J Natl Cancer Inst 1992;84(7):489-94.

Suramin is a polysulfonated urea recently tested in clinical trials as an anticancer agent. To define tumor types for further clinical testing of suramin, the in vitro activity of suramin was assessed against fresh human tumor specimens. Inhibition of tumor colony formation (human tumor clonogenic assay [HTCA] method) and inhibition of tritiated thymidine incorporation (TTI method) were used as indicators of drug sensitivity of the tumors. However, when these assay methods were directly compared against melanoma and ovarian cancer specimens, individual tumors were generally more sensitive to suramin (greater inhibition relative to control) using the HTCA method, whereas the TTI method appeared to underestimate suramin's antitumor activity. These in vitro results provide an basis for experimental clinical evaluations of suramin therapy in patients with colon, endometrial, kidney, non-small-cell lung, and ovarian cancers as well as malignant melanoma and mesothelioma.

7
Brusick DJ. CARCINOGENICITY THE USE OF ANIMAL MODELS AND SHORT-TERM PREDICTIVE TESTS. Frazier, J. M. (ED.). IN VITRO TOXICITY TESTING: APPLICATIONS TO SAFETY EVALUATION. VIII+299P. Marcel Dekker, Inc.: New York, New York, USA; Basel, Switzerland. Illus. ISBN 0-8247-8614-9. 1992;0(0):221-244.

No abstract.

CELL CULTURE
8
Gaudreau P, Boulanger L, Abribat T. AFFINITY OF HUMAN GROWTH HORMONE-RELEASING FACTOR (1-29)NH2 ANALOGS FOR GRF BINDING SITES IN RAT ADENOPITUITARY. J Med Chem 1992;35(10):1864-9.

Previous research on growth hormone-releasing factor analogs used pituitary cell culture assay systems to evaluate in vitro their biological activity. However,

binding assay systems in which receptor affinity and peptide stability can be assessed independently have been lacking. The authors have recently developed a sensitive GRF binding assay with [125I-Tyr10]hGRF(1-44)NH2, this method was applied to structure- affinity studies as a first step of screening GRF analogs. Acylation of the N-terminus of hGRF(1-29)NH2 generally decreased its affinity (relative affinity to hGRF(1-29)NH2 (RA), 26-85%). Replacement of the C-terminal carboxamide by a free carboxylic function decreased affinity likely by diminishing its proteolytic stability (RA, 57%). Removal of Tyr1, Ser9, Lys12, Val13, Gly15, Gln16, or Lys21 decreased its affinity (RA, <3%). Multiple amino acid deletions in the segment 13-21 of hGRF(1-29)NH2 also led to a loss of affinity as did replacing segment 13-15, 16-18, or 19-21 by an octanoyl moiety (RA, <1%). Removal of Asn8, Gln24, Asp25, Ile26, Met27, and Ser28 or Arg29 had less effect on GRF receptor affinity (RA, 5-33%). Removal of Met27 or Ser28 only slightly affects hGRF(1-29)NH2 affinity (RA, 62-78%). Altogether, these results indicate that the amino acids contained in the segment 13-21 are more important than those of 24-29 to insure high affinity receptor binding or to maintain an optimal conformation to allow GRF binding.

9
He L, Gupta D. THE ROLE OF ACTH AND CRH IN REGULATING HUMAN PERIPHERAL MONONUCLEAR CELL RESPONSES IN VITRO. Neuroendocrinol. Lett 1991;13(6):443-50.

The immune system is known to be integrated with other physiological circuits, such as the central nervous system and the neuroendocrine system. Prepared for this study were peripheral mononuclear cells (PMNC) from blood obtained from healthy adult donors. When the isolated PMNCs were tested with ConA or PHA as mitogens, [3H]thymidine incorporation was stimulated in a dose-dependent fashion. However, when ACTH was added, whether simultaneously or 48-h after cultivation, no difference was observed between control and test groups. ACTH-releasing hormone (CRH), on the other hand, stimulated lymphocyte proliferation when added simultaneously with ConA. The failure of ACTH to stimulate proliferation may be due to the currently used human PMNCs of which only 10-15% are B cells. Thus, ACTH probably does not stimulate all lymphocyte proliferation.

10
Wobus AM, Wallukat G, Hescheler J. PLURIPOTENT MOUSE EMBRYONIC STEM CELLS ARE ABLE TO DIFFERENTIATE INTO CARDIOMYOCYTES EXPRESSING CHRONOTROPIC RESPONSES TO ADRENERGIC AND CHOLINERGIC AGENTS AND CALCIUM CHANNEL BLOCKERS. Differentiation (Berlin) 1991;48(3):173-82.

A defined cultivation system was developed for the differentiation of pluri-potent embryonic stem cells of the mouse into spontaneously beating cardiomyocytes, allowing investigations of chronotropic responses, as well as electrophysiological studies of different cardioactive drugs in vitro. The cellular system described may prove useful as an in vitro assay method for toxicological investigations of chronotropic drugs and a model system for studying commitment and cellular differentiation in vitro.

11
Wang E, Gu W, Huang W, Zhou Y, Wang R, Fu A, Wang J. AN IN VITRO DRUG SENSITIVITY TEST USING A HIGHER 3H-TdR INCORPORATION AND A MODIFIED HUMAN TUMOR STEM CELL ASSAY. Shanghai Dier Yike Daxue Xuebao 1991;11(4):281-4.

An in vitro drug sensitivity test was developed to evaluate the lethal effects of drugs on human pulmonary carcinoma cells (HPCC). This method is a variant and combination of Human Tumor Stem Cell Assay (HTSCA) and as a short-term test using [3H]TdR (thymidine) incorporation may be useful to oncology clinics.

12
Abdel-malek Z, Swope VB, Pallas J, Krug K, Nordlund JJ. MITOGENIC, MELANOGENIC, AND cAMP RESPONSES OF CULTURED NEONATAL HUMAN MELANOCYTES TO COMMONLY USED MITOGENS. J CELL Physiol 1992;150(2):416-425.

The following studies have been undertaken to compare and correlate the effects of 12-0-tetradecanoylphorbol acetate (TPA), basic fibroblast growth factor (bFGF), cholera toxin (CT), and isobutyl methylxanthine (IBMX) on neonatal human melanocyte (NHM) proliferation, tyrosinase activity, and cyclic adenosine monophosphate (cAMP) concentration. NHM proliferation at a maximal rate in medium containing 8 nM TPA, 200 n/ml CT, and 10-4 M IBMX. TPA alone did not result in optimal melanocyte proliferation, and, as previously shown, its mitogenic effect was greatly enhanced by the addition of CT and IBMX individually or concomitantly. Human

recombinant (hr) bFGF could replace TPA in the NHM growth medium. The mitogenic effect of 1.2 ng/ml hrbFGF was potentiated in the concomitant but not individual presence of CT and IBMX. TPA alone in the absence of CT and IBMX caused a dose-dependent stimulation of tyrosinase activity. Maximal tyrosinase activity was obtained in the presence of 0.8 nM TPA, 20 ng/ml CT, and 10-4 M IBMX. Unlike TPA, hrbFGF alone resulted in inhibition of tyrosinase activity. In the presence of hrbFGF, tyrosinase activity was potentiated by CT and IBMX, but not by CT alone. Neither TPA nor hrbFGF alone could increase intracellular cAMP levels. Further studies on NHM will be aimed at determining the exact role of protein kinase C (PKC) in regulating proliferation and melanogenesis and the mechanism(s) activated by hrbFGF.

13
Rofstad EK. RETENTION OF CELLULAR RADIATION SENSITIVITY IN CELL AND XENOGRAFT LINES ESTABLISHED FROM HUMAN MELANOMA SURGICAL SPECIMENS. Cancer Res 1992;52(7):1764-9.

Six human melanoma xenograft lines derived from metastatic lesions in six patients have been established in athymic mice. Permanent cell lines in monolayer culture have been established from four of the xenograft lines. The cellular radiation sensitivity of the donor patients' tumors, the xenograft lines, and the cell lines were measured in vitro. The cell and xenograft lines have growth properties in vitro and in vivo that render a wide variety of experiments possible. Consequently, they show promise for future studies of human tumor radiation biology.

14
Tsuchiya T. METHOD OF MICROMASS CULTURE OF MIDBRAIN CELLS AND ITS APPLICATION TO IN-VITRO MECHANISTIC STUDY. Thirty-first Annual Meeting of the Japanese Teratology Society, Izumo, Japan, July 11-12, 1991. Teratology 1991;44(6):7B.

No abstract.

15
Ruchaud S, Boiron O, Cicolella A, Lanotte M. ETHYLENE GLYCOL ETHERS AS HEMOPOIETIC TOXINS--IN VITRO STUDIES OF ACUTE EXPOSURE. Leukemia 1992;6(4):328-34.

Ethylene glycol ethers and their acetate derivatives were analyzed for their toxicity in vitro on several hemopoietic cell lines, either growth-factor-dependent or leukemic, in mouse, rat, and human species. The possibility that fibroblast or macrophage cells worked on the detoxification of the culture is suggested. Results are

discussed with regard to epidemiological and in vivo experimental data presently available.

16
Naughton BA, Sibanda B, Triglia D, Naughton GK. RAT BONE-MARROW CELL PROLIFERATION AND DIFFERENTIATION AS AN INDEX OF THE EFFECTS OF XENOBIOTICS IN VITRO. Toxicol in Vitro 1991;5(5-6):389-94.

A three-dimensional bone marrow culture system was utilized for assessing the toxicity of various chemotherapeutic agents. This model, which exhibits multilineage hematopoiesis and promotes the growth of progenitor cells for extended periods, was treated with various concentrations of Ara-C, cyclophosphamide (CP), methotrexate (MTX), or 5-fluorouracil (5-FU). The three-dimensional cultures may be useful as substrates for toxicity testing as they contain all the cell types present in vivo, are physiological with respect to their growth patterns, are easily manipulable, and can be maintained for extended periods of time.

17
Lasarow RM, Isseroff RR, Gomez EC. QUANTITATIVE IN VITRO ASSESSMENT OF PHOTOTOXICITY BY A FIBROBLAST-NEUTRAL RED ASSAY. J Invest Dermatol 1992;98(5):725-9.

Adapted was the neutral red uptake assay for quantitative assessment of injury to fibroblast cultures by potential phototoxins. Tetracycline derivatives, quinolone derivatives, and chlorpromazine were used as model compounds for development of the assay. Human fibroblasts were incubated with potential phototoxins, the cell cultures irradiated with UV, and the capacity for neutral red uptake determined. An additional group of phototoxic drugs, quinolone antibacterials, were studied. Nalidixic acid, ofloxacin, ciprofloxacin, and norfloxacin all demonstrated phototoxicity, with nalidixic acid showing the greatest decrease in neutral red uptake. This

methodology may provide a useful, rapid method to quantify phototoxic potential of new drugs or suspected phototoxins.

18
Choi J, Suzuki K, Takamata T, Fukuyo S.
BIOCOMPATIBILITY OF THE URETHANE SHAPE MEMORY POLYMER. [1]. TOXICITY SCREENING USING THE AGAR OVERLAY TEST.
Matsumoto Shigaku 1992;17(2):182-8.

In vitro testing methods using cell culture have become common in the evaluation of the biological effects of dental material. One of these tests is an in vitro test using a human cell culture as means to determine the general cytotoxic effect of the urethane shape memory polymer. The Agar Overlay Test (AOT) was used for the cytotoxicity screening of both the urethane shape memory polymer and heat-polymerized denture base polymers. Tests indicated that heat-polymerized denature base polymers have some effect on the AOT culture cells but the urethane shape memory polymer has potential utility as a dental material.

19
Brown RC, Hoskins JA, Sara EA, Cole K, Evans CE.
IN-VITRO METHODS TO DETERMINE THE BIOLOGICAL ACTIVITIES OF PARTICULATE MINERAL POLLUTANTS. Sixth International Workshop on In-vitro Toxicology, Domaine de Saillac.

France, October 1-6, 1990. Toxicol in Vitro 1991;
5(5-6):493-498.

No abstract.

20
Crespi CL. EXPRESSION OF CYTOCHROME P450 cDNAs IN HUMAN B LYMPHOBLASTOID CELLS: APPLICATIONS TO TOXICOLOGY AND METABOLITE ANALYSIS. Methods Enzymol 1991;
206(Cytochrome P450):123-9.

This research focused on the development of human cell systems for toxicological applications in general and gene locus mutation assays in particular. Compared to nonhuman in vitro systems, human cell systems have the potential to be better models of human susceptibility to the toxic effects of environmental compounds. The key to the development of such systems has been the expression of xenobiotic metabolism in human cell

culture. After attempting a variety of traditional approaches to increase cellular capacity to metabolize xenobiotics and achieving limited success with these methods, an approach based on cDNA transfection for the introduction of specific human xenobiotic-metabolizing enzymes into human cells has been developed. The AHH-1 TK+/- human cell line, in conjunction with extrachromosomal vector-mediated human cytochrome P 450 cDNA transfection, was used to construct human cell lines containing specific capacities to metabolize xenobiotics. Properties of the AHH-1 TK+/- cell line allowed analysis of the effects of specific human P 450 expression on metabolite production, chemically induced cell death, and chemically induced genetic damage.

21
Guzzie PJ, Oshiro Y, Soelter S, Balwierz PS, Young RR, Gad SC, Piper CE. SELECTION AND EVALUATION OF AN APPROPRIATE MODEL FOR SCREENING ADENOSINE ANALOGS FOR CHROMOSOME ABERRATIONS. Toxicol Meth 1991;1(3):172-87.

As part of a preclinical safety assessment profile, the adenosine analog SC-46256 (I), a candidate antihypertensive drug, was tested for potential genotoxicity in a series of in vitro and in vivo assays. Negative results were obtained in the Salmonella/microsome and CHO/HGPRT point mutation assays and in an acute mouse micronucleus test. However, in the presence of exogenous metabolism, positive results in the mouse lymphoma L5178Y assay and an in vitro chromosome aberration assay with purified rat lymphocytes indicated that the analog was a potential clastogen. Both adenosine and the analog were subsequently tested in rat lymphocytes using whole-blood vs. purified lymphocyte cultures. High concentrations of adenosine and the adenosine analog induced chromosome aberrations in the purified lymphocytes. However, neither compound induced chromosome damage when cultured in the presence of whole blood, regardless of the presence or absence of the metabolic activation system. Results demonstrate the importance of culture conditions when designing in vitro test protocols. This study exemplifies the need to explore the mechanisms of activity as well as in vivo relevance of in vitro results.

22
Voss J-U, Seibert H. MICROCARRIER-ATTACHED RAT HEPATOCYTES AS A XENOBIOTIC-METABOLIZING SYSTEM IN

COCULTURES. Cell Biol Toxicol 1991;7(4):387-400.

A method for the primary culture of rat liver cells on collagen-coated dextran microcarriers is described. Microcarrier-attached hepatocytes were co-cultured with BALB/c 3T3 cells to study the metabolism-mediated cytotoxicity of cyclophosphamide (CPA). In co-culture with hepatocytes, cytotoxicity of CPA was expressed in a time- and concentration-dependent manner. Results indicate that co-cultivation of microcarrier-attached rat liver cells with target cells represents an approach to the study of the metabolism-mediated toxicity of xenobiotics in vitro.

23
Janecki A, Jakubowiak A, Steinberger A. EFFECT OF CADMIUM CHLORIDE ON TRANSEPITHELIAL ELECTRICAL RESISTANCE OF SERTOLI CELL MONOLAYERS IN TWO-COMPARTMENT CULTURES: A NEW MODEL FOR TOXICOLOGICAL INVESTIGATIONS OF THE "BLOOD-TESTIS" BARRIER IN VITRO. Toxicol Appl Pharmacol 1992;112(1):51-57.

The effect of various doses (0.75-24 muM) of cadmium chloride on the development of intercellular tight junctions by immature rat Sertoli cells (Sc) was investigated in vitro using the two-compartment culture system. The status of tight junctions was monitored by repeated measurements of the transepithelial electrical resistance (TER). For defining the specificity of cadmium chloride effects, the TER changes were correlated with Sc secretory activity (immunoactive inhibin), the cell number (DNA content), and viability (MTT test). The effects of this cadmium compound depended on the concentration of the toxicant as well as on the onset and duration of exposure (4 and 18 hr on Day 1 or 5 of culture). It is believed that the experimental model and approach reported in this paper should be useful for investigating the mechanism of action of known or potential testicular toxicants, particularly those suspected to compromise the integrity of the "blood-testis" barrier.

24
Laflamme D, Faustman E. ROLE OF TRANSFORMING GROWTH FACTOR-BETA IN ORGANOGENESIS: IN VITRO INVESTIGATION USING LIMB AND MIDBRAIN CELLS. Toxicologist 1990 Feb;10(1):275.

Growth factors have been identified as important modulators of cellular growth and differentiation and alteration of these factors has been proposed as a mechanism for developmental toxicity. The aim of these studies is to understand the role of transforming growth factor-beta(TGFbeta-1) in differentiation. For this purpose the authors used the differentiating micromass rat embryo midbrain (CNS) and limb bud (LB) primary culture systems. TGFbeta-1 is added to the cultures 2 hours after plating on day 0 and differentiation and cytotoxicity is evaluated on day 5. In these cultures, the exogenous addition of TGFbeta-1 seems to selectively inhibit differentiation of cell types. In other systems, the effects of TGFbeta-1 have been shown to be multi-functional depending on concentration, location, growth conditions and timing. This study of growth factor effects represents a further characterization of these widely used cell systems.

25
Rumney CJ, Rowland IR. IN VIVO AND IN VITRO MODELS OF THE HUMAN COLONIC FLORA. Crit Rev Food Sci Nutr 1992; 31(4):299-331.

The study of colonic flora composition and metabolism presents considerable methodological problems. Attempts to circumvent these problems have led to the development of numerous in vitro and in vivo models to simulate the human colon and its microbial population. As in vivo models, conventional laboratory animals have limitations. Data of greater relevance to humans can be obtained by using germ-free rodents as carriers of human colonic bacteria. The use of such models to study the toxicity of chemicals and gastro-intestinal infections are discussed. Advantages and disadvantages of various in vitro systems for studying gut microflora and associated metabolic activity (from simple static cultures to the more sophisticated continous and semicotinuous flow models) are reviewed. Final sections of the review are devoted to the major applications (current and future) of the models, including fermentation studies on dietary fiber, metabolism of nutrients and foreign compounds (including carcinogens) in food, and the investigation of colonization resistance.

26
Ching LM, Finlay GJ, Joseph WR, Baguley BC. IN VITRO METHODS FOR SCREENING AGENTS WITH AN INDIRECT MECHANISM OF AMTITUMOR ACTIVITY: XANTHENONE ANALOGS OF FLAVONE

ACETIC ACID. Eur J Cancer 1991;27(12):1684-9.

Xanthenone-4-acetic acid (XAA) resembles flavone acetic acid (FAA) in its effects on solid tumors in mice. The activity of methyl-substituted XAA derivatives in vitro was determined using 18-h 1Cr-release assays, continuous exposure growth inhibition assays, and stimulation of tumoricidal activity of cultured murine resident peritoneal macrophages. In vitro immune stimulation may be more appropriate than direct cytotoxicity for screening compounds with indirect mechanisms of antitumor activity.

27
Shah VP, Elkins J, Skelly JP. RELATIONSHIP BETWEEN IN VIVO SKIN BLANCHING AND IN VITRO RELEASE RATE FOR BETAMETHASONE VALERATE CREAMS. J Pharm Sci 1992; 81(Jan):104-106. (9 REFS)

Two brands of betamethasone valerate cream were evaluated to examine the relation between skin blanching effects in healthy volunteers and drug release rates in vitro, as determined by using a diffusion cell system and a synthetic membrane. One cream formulation exhibited significantly higher blanching than the other product. The drug release rate was higher for the higher blanching formulation and was statistically different from the other product. The method for determining the drug release rate was found to be simple, reliable, and reproducible. It was concluded that the diffusion cell system and synthetic membrane for determining in vitro drug release is

useful for evaluating the in vivo blanching effects of betamethasone.

CYTOGENETICS
28
Hadnagy W, Seemayer NH. IN-VITRO CYTOGENETIC ASSAYS FOR THE DETECTION OF MITOTIC ANEUPLOIDY BY PARTICULATE POLLUTANTS. Sixth Interantional Workshop on In-vitro Toxiclolgy, Domaine de Saillac, France. October 1-6, 1990. Toxicol in Vitro 1991;5(5-6):507-510.

No abstract.

29
Roloff BD, Belluck DA, Meisner LF. CYTOGENETIC STUDIES

OF HERBICIDE INTERACTIONS IN VITRO AND IN VIVO USING ATRAZINE AND LINURON. Arch Environ Contam Toxicol 1992;22(3):267-271.

The herbicides atrazine and linuron, found in Wisconsin's groundwater, were tested alone and in combination, both in vivo and in vitro, to determine their individual and combined genotoxic effects. Human lymphocytes exposed in vitro to either 1 mug/ml linuron or 0.001 mug/ml atrazine showed little chromosome damage, whereas significant chromosome damage was observed in lymphocytes simultaneously exposed to 0.5 mug/ml linuron and 0.0005 mug/ml atrazine, suggesting at least an additive model. In another experiment, mice were fed 20 mug/ml atrazine, 10 mug/ml linuron, or a combination of 10 mug/ml atrazine and 5 mug/ml linuron in their drinking water for 90 days, after which bone marrow cells and cultured splenocytes were examined for chromosomal damage. None of the treatment groups showed chromosome damage in bone marrow whereas the cultured splenocytes demonstated damage in all treatment groups. The experiments suggest that, prior to assessing the risk of a herbicide, it may be compulsory to test it in combinations which mimic the mixtures which would occur under field conditions, such as in contaminated groundwater.

30
Johnston TE, Umbenhauer DR, Bear KL, Dysart GR, Laws GM, Adams SP, Grossman S JG, Galloway SM. ASSESSMENT OF HUMAN LIVER S-9 FOR USE IN IN-VITRO CYTOGENETIC ASSAYS. 23RD Annual Scientific Meeting of the Environmental Mutagen Society, Reno/Sparks, Nevada, USA, March 15-19, 1992. Environ Mol Mutagen Suppl 1992;0(20):27.

No abstract.

CYTOTOXICITY
31
Bassi AM, Piana S, Penco S, Bosco O, Brenci S, Ferro M. USE OF AN ESTABLISHED CELL LINE IN THE EVALUATION OF THE CYTOTOXIC EFFECTS OF VARIOUS CHEMICALS. Boll Soc Ital Biol Sper 1991 Aug;67(8):809-16.

The HTC hepatoma cell line was used as an "in vitro" model to detect the cytotoxicity of eighteen chemicals, chosen on the basis of different biological activities and physicochemical characteristics. Two different cytotoxicity assays measuring cell lethality (CS) or inhibition of cell growth (CF) were developed using confluent cell monolayers

or to colony-forming cells, respectively. Cells were exposed to the chemicals at doses ranging from 10(-6) M to 10(-2) M for 24 hours. The CF test sensitivity being greater than that of the CS test. A battery of cytotoxicity tests could be established to offer simple, rapid and economic methods as complementary and, in part, alternatives to the use of laboratory animals.

32
Bierbaum PJ, Peters JM. PROCEEDINGS OF THE SCIENTIFIC WORKSHOP ON THE HEALTH EFFECTS OF ELECTRIC AND MAGNETIC FIELDS ON WORKERS. Held in Cincinnati, Ohio on January 30-31, 1991. Govt Reports Announcements & Index (GRA&I), Issue 09, 1992.

Workshop participants discussed various aspects of the health effects of worker exposure to electric and magnetic fields and means to assess such effects. Topics discussed included low frequency electromagnetic fields, biological effects of extremely low frequency electromagnetic fields, health effects of exposures, occupational exposure assessment for electric and magnetic fields in the 10 to 1000 hertz frequency range, and magnetic field management. Research recommendations from workshop panels concerning in-vitro/cellular mechanism studies, epidemiologic studies, exposure assessments, and methods for reducing exposures were provided. See also Bibliography, PB91-173351.

33
Lash LH, Zalups RK. MERCURIC CHLORIDE-INDUCED CYTOTOXICITY AND COMPENSATORY HYPERTROPHY IN RAT KIDNEY PROXIMAL TUBULAR CELLS. J Pharmacol Exp Ther 1992; 261(2):819-29.

Previous work showed that uninephrectomized (NPX) rats are more susceptible to the nephropathy induced by some doses of HgCl2 than sham-operated (SHAM) rats. The aim of the present study was to investigate the cytotoxic effects of HgCl2 in proximal tubular (PT) cells isolated from the kidney(s) of both NPX and SHAM rats. The study was designed to test if isolated PT cells that have undergone compensatory hypertrophy in vivo are more sensitive to the cytotoxic effects of HgCl2 in vitro than PT cells isolated from the kidneys of control animals. PT cells were purified from suspensions of renal cortical cells by Percoll density gradient centrifugation. Results demonstrate the

usefulness of freshly isolated PT cells from NPX rats as an in vitro model system useful in investigating the biochemical mechanisms by which compensatory renal growth alters susceptibility to chemical-induced nephrotoxicity.

34
Chu J, Yang A, Wang B, Zhang H. THE EFFECT OF A MIXTURE OF IMMUNOTOXINS CONTAINING PAP-S AND THREE MONOCLONAL ANTIBODIES TO HUMAN T-LYMPHOCYTES: CYTOTOXIC TO HUMAN LEUKEMIC CELLS IN VITRO. Tongji Yike Daxue Xuebao 1991;20(6):365-8.

In this study a mixture of immunotoxins contg. pokeweed antiviral protein (PAP-S) and three monoclonal antibodies (McAb) against human T-lymphocytes was used for in vitro eradication of leukemic cells. Immunotoxins may be useful in the in vitro eradication of leukemic cells as well as for T-cell prophylaxis against graft vs. host disease in allogenic bone marrow transplantation.

35
Safrit JT, Bonavida B. HIERARCHY OF IN VITRO SENSITIVITY AND RESISTANCE OF TUMOR CELLS TO CYTOTOXIC EFFECTOR CELLS, CYTOKINES, DRUGS AND TOXINS. Cancer Immunol Immunother 1992;34(5):321-8.

Tumor cell drug resistence has led to the development of therapeutic modalities which include biological response modifiers, lymphokine-activated killer cells (LAK), and cytokines alone and in combination. The premise of these modalities is that drug resistance can be overcome by other cytotoxic agents or cytotoxic effector cells. However, the relationship between tumor cell sensitivity to these different agents and the cytotoxicity caused by drugs is not well understood. Thus, understanding the relationship between these systems to assess tumor cell cytotoxicity is essential for optimal therapeutic intervention. To this end, the authors compared the tumor cell cytotoxicity mediated by recombinant tumor necrosis factor (rTNF), cytotoxic effector cells (natural killer cells, monocytes, LAK cells), chemotherapeutic drugs, and microbial toxins. Human tumor cell lines sensitive and resistant to rTNF or drugs were used to evaluate the effectiveness of the other cytotoxic modalities in vitro. From a majority of cell lines resistant to rTNF to cell minority lines resistant to LAK, the authors found an interesting gradation of sensitivity and/or resistance to the other cytotoxic

modalities employed. The hypothesis of an underlying common mechanism of action within these in vitro systems is discussed.

36
Kakiuchi H, Imai K. IN VITRO CYTOTOXICITY OF DENTURE ADHESIVES. Shika Zairyo Kikai 1990;9(2):146-58.

Cell recovery from damage induced by various commercial denture adhesives (three powder types, three rubber types, two cream types and one tape type) was studied in vitro, using 3 tissue culture cell lines (L-929 cells, HEp-2 cells and Gin-1 cells). After cultivation with a denture adhesive for two hours, the cells were transferred to a normal culture environment, and their growth evaluated. The discrepancy among the results obtained by the different methods was considered to be due to the state of the specimen and differences in testing conditions which indicates the importance of selecting an appropriate testing method.

37
Gao M, Binks SP, Chipman JK, Levy LS, Braithwaite RA, Brown SS. INDUCTION OF DNA STRAND BREAKS IN PERIPHERAL LYMPHOCYTES BY SOLUBLE CHROMIUM COMPOUNDS. Hum Exp Toxicol 1992;11(2):77-82.

Incubation of human lymphocytes with sodium dichromate (CrVI) at 37 degrees C for 3 hours resulted in a dose-dependent increase in DNA strand breaks without concurrent cytotoxicity. In contrast, chromium acetate hydroxide (CrIII) failed to induce DNA strand breaks at sub-cytotoxic concentrations. Results indicate that fluorometric analysis of DNA unwinding (FADU) in peripheral lymphocytes might be a method of measuring a biological effect of chromium in occupationally-exposed workers.

38
Psarras V, Wennberg A, Derand T. CYTOTOXICITY OF CORRODED GALLIUM AND DENTAL AMALGAM ALLOYS: AN IN VITRO STUDY. Acta Odontol Scand 1992;50(1):31-6.

The cytotoxicity of one gallium and three different dental amalgam alloys was assessed in a cell culture system. Two evaluation methods were used, the filter method and an extraction method. While differences were found between the two testing methods, no major differences in cytotoxicity were found between the Ga

alloy and the amalgams. The results encourage further study and development of the Ga alloy, which is mercury-free.

39
Aleo MD, Taub ML, Kostyniak PJ. PRIMARY CULTURES OF RABBIT RENAL PROXIMAL TUBULE CELLS: III. COMPARATIVE CYTOTOXICITY OF INORGANIC AND ORGANIC MERCURY. Toxicol Appl Pharmacol 1992;112(2):310-317.

This study further developed primary cultures of rabbit renal proximal tubule cells (RPTC) as an in vitro model applicable to the study of chemical-induced toxicity by investigating the comparative cytotoxicity of mercuric chloride (HgCl2) and methyl mercury chloride (CH3HgCl) to RPTC. This effort demonstrated that rabbit RPTC in primary culture were a useful in vitro model for studying chemical-induced toxicity on a cellular level.

40
Adamis Z, Krass BK. STUDIES ON THE CYTOTOXICITY OF CERAMIC RESPIRABLE DUSTS USING IN VITRO AND IN VIVO TEST SYSTEMS. Ann Occup Hyg 1991;35(5):469-83.

The effect of a number of ceramic raw materials and airborne samples in workplaces has been investigated in vitro [erythrocyte haemolysis, macrophage TTC (2,3,5-triphenyl-tetrazolium chloride) reduction and LDH (lactate dehydrogenase) activity] and in vivo (protein, LDH and phospholipid in cell-free bronchopulmonary lavage). In the in vitro experiments described it was possible to distinguish between the dusts causing different types of reaction in the lung. One practical feature of the test systems used is that the in vitro experiments required only 15 mg of dust, and the in vivo experiments only 100 mg.

41
Dorr RT, Shipp NG, Lee KM. COMPARISON OF CYTOTOXICITY IN HEART CELLS AND TUMOR CELLS EXPOSED TO DNA INTERCALATING AGENTS IN VITRO. Anticancer Drugs 1991;2(1):27-33.

A new approach to antitumor analog selection was evaluated using in vitro cytotoxicity assays in tumor cells and heart cells. Eight anthracycline antibiotics and five non-anthracycline DNA intercalating agents were separately exposed to human 8226 myeloma cells and neonatal rat heart myocytes in vitro. Survival was measured after six

days of culture by the MTT dye method for tumor cells and by ATP content for heart cells. Inhibitory drug concentrations in 50% of cells (IC50) were determined from log-linear dose-response curves for each agent. The results indicated that simultaneous comparisons of cytotoxicity in heart cells and tumor cells can identify agents such as daunorubicin and mitoxantrone which are known to produce less cardiac toxicity in vivo. on further assessment this methodology may be applicable to preclinical screening programs to select active DNA intercalating agents with low cardiotoxic potential.

42
Janz S, Shacter E. MOLECULAR ENCAPSULATION AND DELIVERY OF ALKANES TO LIVING MAMMALIAN CELLS FOR RISK ASSESSMENT AND PHARMACEUTICAL APPLICATIONS. Govt Reports Announcements & Index (GRA&I), Issue 04, 1992.

A complex of a cyclodextrin and an alkane, alkene, alkyne, aromatic compound, etc. can be prepared according to the method of the invention. These complexes can be delivered to prokaryotic and eukaryotic cells, tissues, and organs in vitro and in vivo. In this manner, the toxic, genotoxic, and mitogenic effects of these compounds can be assessed.

43
Sasaki K, Tanaka N, Watanabe M, Yamada M. COMPARISON OF CYTOTOXIC EFFECTS OF CHEMICALS IN FOUR DIFFERENT CELL TYPES. Sixth Interantional Workshop on In-vitro Toxicology, Domaine de Saillac. France, October 1-6, 1990. Toxicol in Vitro 1991;5(5-6):403-406.

No abstract.

44
Ikarashi Y, Toyoda K, Ohsawa N, Uchima T, Tsuchiya T, Kaniwa M-A, Sato M, Takahashi M, Nakamura A. COMPARATIVE STUDIES BY CELL CULTURE AND IN VIVO IMPLANTATION TEST ON THE TOXICITY OF NATURAL RUBBER LATEX MATERIALS. J Biomed Mater Res 1992;26(3):339-356.

Colony assay using V79 cells, the agar diffusion assay with L929 cells, and the 7-day rabbit muscle implantation test were employed to evaluate the cytotoxicity and tissue toxicity of natural rubber latex (NRL) materials. This in vivo implantation test

showed that, among 13 histological parameters, thickness of inflammatory layer was the most useful index to evaluate tissue responses quantitatively. From the results, it appears that the colony assay provides a more reliable prediction of tissue response than the agar diffusion assay.

45
Vitiello S, Cadic C, Dupuy B. IN VITRO STUDY OF THE CYTOTOXICITY OF INFLAMMATION MEDIATORS SECRETED AROUND MICROENCAPSULATED CELLS. C R Seances Soc Biol Fil 1991; 185(5):338-44.

One of the implantation problems that can be identified in immunoprotected living cells is the appearance of local inflammatory phenomena around microcapsules. Some of the mediators released in such pathophysiological conditions were tested. A toxic action of compounds such as elastase, collagenase was evidenced. Interleukins 1 and 2 revealed no cytotoxicity within the test limits on the experimental cellular model chosen. Results underline the importance of inflammatory mediators released by adjacent cells of the implant.

46
Jover R, Ponsoda X, Castell JV, Gomez-Lechon MJ. EVALUATION OF THE CYTOTOXICITY OF TEN CHEMICALS ON HUMAN CULTURED HEPATOCYTES: PREDICTABILITY OF HUMAN TOXICITY AND COMPARISON WITH RODENT CELL CULTURE SYSTEMS. Toxicol In Vitro 1992;6(1):47-52.

The cytotoxic effect of 10 chemicals on the MEIC list (evaluated in the Multicentre Evaluation of In Vitro Cytotoxicity organized by the Scandinavian Society of Cell Toxicology) was evaluated using human and rat cultured hepatocytes and in the non-hepatic murine 3T3 cell line. The MTT test was used as an endpoint to evaluate cytotoxicity after 24 hr of exposure to the chemicals. The predictability of human toxicity using human hepatocytes was analysed and compared with results obtained using rodent cell culture systems and rat and mouse LD50 tests. The data suggest that for the 10 chemicals tested, acute toxicity in humans was more accurately predicted using human hepatocytes than using rat hepatocytes or mouse non-hepatic 3T3 cells.

47
Patel BC, Courtney JM, Evans JH, Paul JP. BIOCOMPATIBILITY

ASSESSMENT: APPLICATION OF FLUORESCENT PROBE RESPONSE (FPR) TECHNIQUE. Biomaterials 1991;12(8):722-6.

An in vitro test procedure capable of discriminating effectively between intact and membrane-damaged cells has been developed. The procedure utilizes fluorescein diacetate and ethidium bromide as fluorescent probes. Properties of the probes and collapse in the selective cytoplasmic membrane permeability barrier of damaged cells have functional cells fluoresce bright green, but membrane-damaged cells fluoresce bright red. Investigations with natural rubber, silicone and acrylic polymers confirmed the suitability of the procedure to distinguish between the biocompatibility of materials on the basis of cytotoxicity.

48
Voss JU, Seibert H. MICROCARRIER-ATTACHED RAT HEPATOCYTES AS A XENOBIOTIC-METABOLIZING SYSTEM IN COCULTURES. Cell Biol Toxicol 1991;7(4):387-99.

A method for the primary culture of rat liver cells on collagen-coated dextran microcarriers is described. Ethoxycoumarin deethylase (EOD) activity 24 hr after inoculation was comparable for liver cells cultured on microcarriers and on collagen-coated dishes. Cells were cultured on microcarriers for up to 48 hr and retained 25% of the initial EOD-activity that was seen in freshly isolated liver cells. Microcarrier-attached hepatocytes were co-cultured with BALB/c 3T3 cells to study the metabolism-mediated cytotoxicity of cyclophosphamide (CPA). Results indicate that co-cultivation of microcarrier-attached rat liver cells with target cells represents an approach to the study of the metabolism-mediated toxicity of xenobiotics in vitro.

49
Laschinski G, Vogel R, Spielmann H. CYTOTOXICITY TEST USING BLASTOCYST-DERIVED EUPLOID EMBRYONAL STEM CELLS: A NEW APPROACH TO IN VITRO TERATOGENESIS SCREENING. Reprod Toxicol 1991;5(1):57-64.

To develop a mammalian in vitro system for teratogenicity testing, cytotoxicity of xenobiotics was evaluated in pluripotent euploid embryonal stem cells (ESC) derived from mouse blastocysts. Only compounds that do not require metabolic activation were selected for testing from the database for validation of in vitro teratogenesis assays by Smith et al. Results obtained with ESC

were compared to corresponding data from fibroblasts from day-14 mouse embryos to detect differences in sensitivity between undifferentiated and differentiated cells. Although some xenobiotics had to be classified as false negatives in this system, the ESC cytotoxicity assay has promise as a new in vitro screening assay in teratology.

50
Mohr KL, Working PK. AN IN VITRO TECHNIQUE TO DETECT DOMINANT LETHAT MUTATIONS INDUCED IN MOUSE OOCYTES BY ETHYL METHANESULPHONATE EXPOSURE IN VIVO. Toxicology In Vitro 1990;4(2):115-21.

Administration of chemical mutagens to the female rodent can induce dominant lethal mutations in oocytes and affect embryo development after fertilization. Traditional in vivo dominant lethal assays cannot separate specific genotoxic effects on the embryo from generalized cytotoxic effects. We have used embryo culture, after in vivo exposure of oocytes, to separate the genotoxic effects of a chemical on oocytes from effects due to maternal toxicity. Pre- and post-implantation development in culture was monitored in embryos recovered at the two-cell stage from females dosed ip, 30-32 hr before ovulation, with 125 or 250 mg/kg ethyl methanesulphonate (EMS)/kg body weight. All stages of development were affected by the EMS treatment. Study results indicate that an in vitro dominant lethal test can be useful in evaluating damage to metaphase-1 oocytes. The in vitro test can be used to study the effects of chemicals on all stages of zygote development thereby separating induced genotoxic effects from the possible effects of maternal toxicity on zygote development.

51
Regan CM, Larsson OM, Martin ML, Schousboe A, Williams DC. IN VITRO SCREENING FOR ANTICONVULSANT-INDUCED TERATOGENESIS: STRUCTURE-ACTIVITY RELATIONSHIPS IN THE BARBITURATE AND BRANCHED CHAIN CARBOXYLIC ACID CLASSES. Toxicology In Vitro 1991;5(1):77-82.

Described is the ability of in vitro antiproliferative and cytotoxicity assay systems to discriminate teratogenic potential among closely related anticonvulsant agents.

52
Ciapetti G, Roda P, Landi L, Facchini A, Pizzoferrato A. IN VITRO METHODS TO EVALUATE METAL-CELL

INTERACTIONS. Int J Artif Organs 1992;15(1):62-6.

The aim of this study was to test different metals, widely employed in constructing prosthetic devices, by in vitro methods. Biological effects of such materials were analyzed through four different assays which used human lymphocytes and granulocytes. The lymphocyte proliferation assay gave quantitative results, while the viability test showed the morphological appearance of the cells correlated well with previous results. NK cytotoxicity and granulocyte chemokinesis tests provided interesting data on leukocyte performance when challenged with metals. The present study adds new basic information on cell behavior when metal products are present in the body, e.g. around devices implanted in human tissues.

53
Cornelis M, Dupont C, Wepierre J. PREDICTION OF EYE IRRITANCY POTENTIAL OF SURFACTANTS BY CYTOTOXICITY TESTS IN VITRO ON CULTURES OF HUMAN SKIN FIBROBLASTS AND KERATINOCYTES. Toxicol in vitro 1992;6(2):119-128.

The cytotoxicity of surfactants was evaluated on cultures of human skin fibroblasts and keratinocytes in order to predict their eye irritancy potential taking into account both immediate cytotoxicity after 2 hr of incubation and delayed cytotoxicity 24 hr after such incubation. The immediate cytotoxicity ranking of the surfactants, evaluated by MTT or neutral red assay after 2 hr of exposure in minimum Eagle's medium (MEM) without or with 10% foetal calf serum (FCS), was identical for both cell types. Keratinocytes were less sensitive than fibroblasts to all surfactants apart from Tween; however, cytotoxicity ranking remained the same for both cell types. No correlation was found between EC50 values of immediate or delayed cytotoxicity, under various experimental conditions, and ocular irritation scores in vivo.

DEVELOPMENTAL TOXICITY
54
Brown NA, Wiger R. COMPARISON OF RAT AND CHICK LIMB BUD MICROMASS CULTURES FOR DEVELOPMENTAL TOXICITY SCREENING. Toxicol in vitro 1992;6(2):101-107.

This study compares the responses of rat and chick limb bud micromas cultures to chemical treatment. Eight chemicals, of diverse structure, potency and mechanism,

were tested, using two endpoints: extractable alcian blue stain as a measure of differentiation to chondrocytes, and extractable neutral red stain as an index of proliferation. Each chemical reduced differentiation and proliferation in a concentration-related manner.

55
Toraason M, Bohrman JS, Kreig E, Combs RD, Willington SE, Zajak W. INHIBITION OF INTERCELLULAR COMMUNICATION IN V79 CELLS BY DEVELOPMENTAL TOXICANTS. Teratology 1990 May;41(5):596.

Inhibition of intercellular communication is proposed to be one of several possible mechanisms of teratogenesis. We tested 36 coded compounds for their effect on intercellular communication in the V79-cell-metabolic-cooperation assay. Test chemicals were selected from a list of 47 compounds recommended for the evaluation of in vitro assays for developmental toxicants (Smith et al., Teratogen, Carcinogen, Mutagen, 3, 461-480, 1983). In the V79 assay, 6-thioguanine (6-TG) is added to co-cultures of 6-TG resistant cells and 6-TG sensitive cells. Increased survival of 6-TG resistant cell colonies indicates inhibited intercellular communication. A separate cytotoxicity assay for each chemical assessed clonal expansion of 6-TG resistant cells. Ten (37%) of the 27 designated developmental toxicants inhibited intercellular communication (Dunnett's test, P less than 0.01). Of the 10, 3 inhibited only at cytotoxic concentrations. Only 1 (11%) of 9 nonteratogens inhibited intercellular communication, and the effect was small, though statistically significant. These results suggest that the V79-cell-metabolic-cooperation assay is not sensitive to a diverse group of developmental toxicants; however, the assay does appear specific for developmental toxicants as there was only a single false positive response.

56
Toraason M, Bohrman JS, Krieg E, Combes RD, Willington SE, Zajac W, Langenbach R. EVALUATION OF THE V79 CELL METABOLIC COOPERATION ASSAY AS A SCREEN IN VITRO FOR DEVELOPMENTAL TOXICANTS. Toxicol In Vitro 1992; 6(2):165-174.

Inhibition of intracellular 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. Chemicals tested were

selected from a list of 47 agents recommended for the evaluation of in vitro assays for developmental toxicants. In addition to testing the effects of chemicals in intercellular communication, a separate cytotoxicity assay determined the concentration of each chemical that inhibited clonal expansion of V79 cells. Test 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.

57
Steele VE, Morrissey RE, Elmore EL, Gurganus-Rocha D, Wilkinson BP, Curren RD, Schmetter BS, Louie AT, Lamb JC 4th, Yang LL. EVALUATION OF TWO IN VITRO ASSAYS TO SCREEN FOR POTENTIAL DEVELOPMENTAL TOXICANTS. Fundam Appl Toxicol 1988 Nov; 11(4):673-84.

To evaluate two in vitro assays for their ability to detect and separate known developmental toxicants and nontoxicants, a series of 44 coded compounds were assayed by two independent laboratories using standardized protocols. The two test systems were (1) the human embryonic palatal mesenchymal cell growth inhibition assay and (2) the mouse ovarian tumor cell attachment inhibition assay. After all compounds were tested and ranked according to the minimum IC50 value (the millimolar concentration of compound which inhibits growth or attachment by 50% compared to the solvent control) from either test. The chemicals were then decoded and in vitro test result concordance with established in vivo animal and human test results was examined over a wide range of concentration levels (above which the in vitro results were called positive and below which they were considered negative). A positive response from either test was defined as a positive in vitro response. All the data collected indicate that the two assays are complimentary and as such the combination of these assays could be useful as a preliminary screen to establish priorities for in vivo developmental toxicity testing.

58
Kavlock RJ, Oglesby LA, Hall LL, Fisher HL, Copeland F, Logsdon T, Ebron-McCoy M. IN VIVO AND IN VITRO STRUCTURE-DOSIMETRY-ACTIVITY RELATIONSHIPS OF

SUBSTITUTED PHENOLS IN DEVELOPMENTAL TOXICITY ASSAYS. Fundam Appl Toxicol 1991 Feb;16(2):225-9.

No abstract.

59
Fort DJ, Bantle JA. ANALYSIS OF THE MECHANISM OF ISONIAZID-INDUCED DEVELOPMENTAL TOXICITY WITH FROM EMBRYO TERATOGENESIS ASSAY: XENOPUS (FETAX). Teratogenesis Carcinog Mutagen 1990;10(6):463-76.

The developmental toxicity of isoniazid (INH) and acetylhydrazide (AH) and isonicotinic acid (INA) were examined using the frog embryo teratogenesis assay-Xenopus (FETAX). Late Xenopus laevis blastulae were exposed to INH, AH, and INA for 96 hours in two separate static-renewal tests with and without the presence of three differently induced metabolic activation systems (MAS). Results suggest that mixed functional oxidase metabolism may alter the developmental toxicity of INH in vitro by producing a more embryo lethal metabolite, but less teratogenic metabolite(s) than INH or AH themselves. Results are indicative of the potential of FETAX for evaluating toxicological mechanisms of teratogenesis in vitro.

60
Newman LM, Johnson EM, Giacobbe RL, Fu LJ. THE IN VITRO ACTIVATION OF CYCLOPHOSPHAMIDE IN THE HYDRA DEVELOPMENTAL TOXICOLOGY ASSAY. Fundam Appl Toxicol 1990 Oct;15(3):488-99.

The proteratogen cyclophosphamide (CP) was tested in the Hydra Assay in the presence and absence of an in vitro metabolic activation package (MAP) consisting of rat hepatic microsomes (0.06 nmol P450/ml), 500 microM NADPH, and 25 microM MgCl. This metabolic system was developed through a series of interrelated biochemical and biological assays to provide maximum cytochrome P450 mixed-function monooxygenase (MFO) metabolic capacity while controlling the inherent toxicity of the hepatic preparation and the attendant cofactors. The addition of metabolic activation capacity to an in vitro assay, while not essential, markedly enhances its utility and breadth of application in developmental toxicity safety evaluations.

61
Daston GP, Baines D, Yonker JE. CHICK EMBRYO NEURAL

RETINA CELL CULTURE AS A SCREEN FOR DEVELOPMENTAL TOXICITY. Toxicol Appl Pharmacol 1991 Jun 15; 109(2):352-66.

An in vitro screen for developmental toxic potential of chemicals using primary cultures of chick embryo neural retina cells is described. The neural retinas of incubation Day 6.5 White Leghorn chick embryos are dissociated into single cells, which are subsequently maintained in a rotating suspension culture. Under normal circumstances, neural retina cells form spheroidal aggregates of a consistent size over the first 24 hr of culture, an event which is dependent on competent cell-cell interactions. Over the remaining 7-day period of culture, cells continue to divide and grow, and differentiation takes place. Each of these developmentally important events--aggregation, growth, and differentiation--is objectively and quantitatively measured as aggregate size and number, aggregate protein content, and glutamine synthetase (a marker of differentiation) activity, respectively. The effects on each developmental endpoint of 22 chemicals, 14 of which have been demonstrated to be developmentally toxic in one or more mammalian species in vivo, and 8 of which are not developmentally toxic, were evaluated. Chemicals were tested up to a concentration of 40 mM, or until marked cyto-lethality was observed. It was also observed that the test agents differentially affect developmental endpoints. Because the assay's endpoints are measured separately and objectively, it may be possible to use the assay to evaluate the effects of test agents on cellular development.

62
Wise LD, Clark RL, Rundell JO, Robertson RT. EXAMINATION OF A RODENT LIMB BUD MICROMASS ASSAY AS A PRESCREEN FOR DEVELOPMENTAL TOXICITY. Teratology 1990 Mar;41(3):341-51.

The mouse limb bud micromass assay is one of many short-term tests proposed as preliminary screens for potential developmental toxicity. Previous efforts to validate this assay have used too few nonteratogens. The purpose of this study was to examine additional compounds, most of which, based on the literature, were perceived to have low potential for developmental toxicity in vivo. The concentration of each of 23 compounds that produced a 50% inhibition (IC50) of radiolabeled thymidine (T) and sulfate (S) incorporation was determined and used to calculate a T/S ratio. The T/S ratio may be a useful measure of developmental hazard, since T incorporation measures toxicity toward a general cell function (DNA synthesis) and

S incorporation measures mainly toxicity toward a developmentally specific cell activity (chondroitin sulfate synthesis). All compounds tested produced T/S ratios of less than 2.0. Since 22 of these 23 compounds are classified as nonteratogens or nonselective developmental toxins in vivo, a low T/S ratio in this in vitro assay system may be capable of discriminating the potential for developmental hazard in vivo.

63
Newman LM, Johnson EM, Paulson R, Ly T, Giacobbe RL. IN VITRO DEVELOPMENTAL TOXICITY SCREENING OF A COMPLEX MIXTURE WITH THE HYDRA ASSAY: SMOKELESS TOBACCO. Teratology 1990 May;41(5):582.

It is generally understood that tobacco smoke results in developmental toxicity both in humans and experimental animals. Smokeless tobacco (ST) has been tested in laboratory animals. Adverse effects were seen in the conceptus at maternally toxic treatment levels (Tera. Carcin. and Muta. 8:81-93, 1988; Teratology 40:483-494, 1989). Because ST is a complex mixture consisting, at least in part, of unknown constituents, it was considered as a good candidate for in vitro testing in the Hydra Assay to determine whether or not this assay could predict the animal-established developmental toxicity hazard-potential of a complex mixture. Conclusion: Testing in experimental animals and in the Hydra Assay both indicate that ST has the ability to produce adverse effects in the conceptus only at, or very near to, exposure levels toxic to the mother. Therefore, if the complex mixture represented by ST had not been tested in experimental animals but only in the Hydra Assay, the in vitro results would have correctly predicted that ST was a co-affective developmental toxicant. It was noted, however, that ST would still merit at least consideration for testing in pregnant animals because of its wide-spread use at undetermined levels of exposure (Teratology 35:405-427, 1987).

64
Newman LM, Johnson EM, Giacobbe RL, Fu LJ. DEVELOPMENTAL TOXICITY EVALUATION OF SEVERAL COSMETIC INGREDIENTS IN THE HYDRA ASSAY. Teratology 1990 May;41(5):582.

The developmental toxicity hazard-potential of six cosmetic products was determined in the in vitro Hydra Assay to supplement available toxicological information concerning

effects, other than developmental, in order to provide an indication of the priority of these compounds for higher level in vivo developmental toxicity testing. Three highly volatile chemicals: amyl acetate, isomyl acetate, and methylene chloride were tested using the closed test method developed for gasses (Teratol. 39:483, 1989) wherein the assay is conducted within vials with teflon-lined screw top caps to prevent outgassing. The remaining chemicals: sorbic acid, potassium sorbate, and steareth-20 were tested by the standard Hydra Assay. All but one ingredient, potassium sorbate, was predicted by the assay to be generally equally or more toxic to adults than to "embryos" and, therefore, to be low-priority chemicals for more elaborate tests.

65
Kimmel GL. IN VITRO ASSAYS IN DEVELOPMENTAL TOXICOLOGY: THEIR POTENTIAL APPLICATION IN RISK ASSESSMENT. In Vitro Methods in Devel Toxic 1990;:163-73.

No abstract.

66
Leber AP, Scott RC, Hodge MC, Johnson D, Krasavage WJ. TRIETHYLENE GLYCOL ETHERS: EVALUATIONS OF IN VITRO ABSORPTION THROUGH HUMAN EPIDERMIS, 21-DAY DERMAL TOXICITY IN RABBITS, AND A DEVELOPMENTAL TOXICITY SCREEN IN RATS. J Am Coll Toxicol 1990;9(5):507-15.

The methyl, ethyl, and butyl ethers of triethylene glycol (TM, TE, AND TB, respectively) were evaluated in three screening studies to assess their potential hazards to humans. Assessments included (1) an in vitro procedure to determine the ability of the materials to penetrate human skin, (2) a 21-day dermal limit test in rabbits to determine potential systemic toxicities, and (3) a screening procedure to evaluate the chemicals' potential to induce developmental toxicity. Results indicate that triethylene glycol methyl, ethyl, and butyl ethers have very low capacities to be absorbed through the skin of exposed individuals, low potentials to produce systemic toxicity following oral or dermal exposures, and do not appear to be selectively toxic to the developing conceptus. The data indicate that triethylene glycol ethers do not exhibit toxicologic profiles comparable to those of the methyl and ethyl ethers of ethylene glycol.

67

Anonymous. IN VITRO DEVELOPMENTAL TOXICITY ASSAYS. Current Issues in Toxicology 1989;0(0):9-68. [Interpretation and Extrapolation of Reproductive Data to Establsh Human Safety Standards]

No abstract.

68
Jensen M, Newman LM, Johnson EM. RE: PROBLEMS IN VALIDATION OF IN VITRO DEVELOPMENTAL TOXICITY ASSAYS [letter; comment]. [Comment on: Fundam Appl Toxicol 1988 Nov;11(4):673-84.] Fundam Appl Toxicol 1989 Nov;13(4):863-5.

No abstract.

69
Kavlock RJ, Greene JA, Kimmel GL, Morrissey RE, Owens E, Rogers JM, Sadler TW, Stack HF, Waters MD, Welsch F. ACTIVITY PROFILES OF DEVELOPMENTAL TOXICITY: DESIGN CONSIDERATIONS AND PILOT IMPLEMENTATION. Teratology 1991 Feb;43(2):159-85.

The available literature was searched for quantitative test results from both in vitro and in vivo assays for developmental toxicity for five model compounds: cyclophosphamide, methotrexate, hydroxyurea, caffeine, and ethylenethiourea. These compounds were chosen on the basis of their extensive utilization in a variety of assay systems for developmental toxicity as evidenced by their representation in the ETIC database (each generally has 100-500 citations encompassing multiple test systems). Nine cellular-based assays, six assays using whole embryos in culture, as well as Segment II and abbreviated exposure tests for mammalian test species are included in the database. For each assay, critical endpoints were identified, each of which was then given a three-letter code, and criteria for extraction of quantitative information were established. The extracted information was placed into a computerized reference file. The information contained in the file can be used to compare qualitative and quantitative results across multiple assay systems, identify data gaps in the literature, evaluate the concordance of the assays, calculate relative potencies, and examine structure-activity relationships.

70
Copeland MF, Kavlock RJ, Gray JA, Rogers EH, Rehnberg

BF. DOSIMETRY OF P-CYANOPHENOL IN AN IN VIVO DEVELOPMENTAL TOXICITY BIOASSAY. Teratology 1990 May;41(5):545.

As part of an effort to characterize the comparative in vitro/in vivo embryonic dosimetry of a series of p-substituted phenols, the maternal and embryonic dosimetry of p-cyanophenol (p-CP) was determined. Timed-pregnant Sprague-Dawley rats received either 0 or 1000 mg/kg of p-CP by gavage on day 11 of gestation. This dose of p-CP induces maternal but not developmental toxicity; however, addition of p-CP to whole embryo culture is embyotoxic. Rats were placed in metabolism cages and sacrificed at 1, 6, 24, and 48 hours after dosing. Maternal liver, kidney, fat, brain, blood, urine and embryos were collected. The difference in embryotoxicity observed between the in vivo and in vitro bioassays is probably related to delivery of p-CP to the embryo. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

71
Fort DJ, James BL, Bantle JA. EVALUATION OF THE DEVELOPMENTAL TOXICITY OF FIVE COMPOUNDS WITH THE FROG EMBRYO TERATOGENESIS ASSAY: XENOPUS (FETAX) AND A METABOLIC ACTIVATION SYSTEM. J Appl Toxicol 1989 Dec;9(6):377-88.

The potential teratogenic hazard of five compounds was evaluated using the Frog Embryo Teratogenesis Assay--Xenopus (FETAX) and a metabolic activation system. Embryos of the South African clawed frog, Xenopus laevis, were exposed to (i) three compounds suspected to be proteratogenic in mammalian test systems--[2-acetylaminofluorene 2-AAF), rifampicin (RA) and benzo[a]pyrene (BP)] for 96 hours; (ii) one compound unaffected by mixed-functional oxidase (MFO) metabolism--ZnSO4; (iii) one compound thought to be inactivated by cytochrome P-450-cytochalasin D (CD). The metabolic activation system consisted of Aroclor 1254-induced rat liver microsomes. Results demonstrate the utility and importance of a MAS for in vitro developmental toxicity screens such as FETAX. Consistent use of a MAS with FETAX should reduce the number of potential false-positive and false-negative test results.

72
Zhang RW, Newman LM, Johnson EM. COMPARISON OF IN VITRO DEVELOPMENTAL TOXICITY HAZARD-POTENTIAL DETECTION ASSAYS IN HYDRA: REGENERATION VERSUS REAGGREGATION. J Am Coll Toxicol 1990 Dec;9(6):649.

Two of the proposed developmental toxicity prescreen assay systems employ the simple coelenterate Hydra attenuata. One method employs intact body segments of the adult hydra (Fd. Chem. Toxicol. 24:651-652, '86) and the other employs artifical "embryos" consisting of randomally reaggregated dissociated terminally differentiated and pluripotent cells of adult hydra (Terat Carcin Mutag 2:263-276, '82.) It has been suggested that the two assays are similar in ability in assessment of developmental toxicity of chemicals. The present study evaluated the system using body segments and compared the efficiency of the two assays to detect selective developmental toxicants. Results from the two assays were not similar. The regeneration assay (using body segments) appears to be ineffective for the prescreening of chemicals for selective developmental toxicity hazard-potential, use of the artificial "embryo" has been shown to agree (90+% accuracy) with published animal studies.

73
Johnson EM, Newman LM, Giacobbi RL. SOME FUNDAMENTAL PROBLEMS WITH IN VITRO DEVELOPMENTAL TOXICITY PRESCREENING ASSAYS AS ILLUSTRATED WITH BEAN SEEDLINGS. Teratology 1990 May;41(5):568.

During the past decade, numerous investigators have sought to provide reliable, and cost effective in vitro developmental toxicity prescreens. Validity

of test data is established by comparison to data from standardized in vivo evaluations. Investigators relied upon the pioneer list of chemicals (Tera. Carcin. and Muta. 3:461-480, 1983) that was itself flawed (Fund. and App. Tox. 13:863-867, 1989). In addition, validation usually was attempted based on "teratogen" vs "nonteratogen" distinctions even though such often are more a function of treatment level, route, species and timing than the nature of the agent. To illustrate the problem, effects of chemicals were examined in a simple, biologically responsive system--bean seed germination and growth. Endpoints of effects on germination were found that separated agents purported to be "teratogens" by some investigators from "nonteratogens" with an accuracy of 73%. This compares favorably with published in vitro assays using +/- designations. The bean seed test illustrates, perhaps, that the primary database with which in vitro prescreen results are compared is inaccurate and/or that +/- designations are unrealistic. It also illustrates the need to generate what should be a gold standard. This

could be based on one or more of the four determinations possible from standard in vivo tests: pattern of effect, NOAEL, DRC, and/or A/D ratio.

74
Friedman M, Rayburn JR, Bantle JA. DEVELOPMENTAL TOXICOLOGY OF POTATO ALKALOIDS IN THE FROG EMBRYO. TERATOGENESIS ASSAY--XENOPUS (FETAX). Food Chem Toxicol 1991 Aug;298:537-47.

Potatoes frequently contain growth inhibitors and toxic compounds including digestive enzyme inhibitors, lectins and glycoalkaloids. The literature suggests that Solanum alkaloids have the ability to induce neurological damage such as spina bifida and other malformations. As part of a programme of improvement in the safety of potatoes using molecular plant genetics and parallel food safety evaluation, the author's evaluated the effect of several potato glycoalkaloids and aglycones in the frog embryo teratogenesis assay--Xenopus (FETAX) with and without metabolic activation by Aroclor 1254-induced rat liver microsomes. The data suggest that the glycoalkaloid alpha-chaconine is teratogenic and more embryotoxic than alpha-solanine. The in vitro teratogenesis assay should be useful for: (a) predicting the teratogenic potential of solanaceae alkaloids, glycoalkaloids and related natural products; and (b) facilitating experimental approaches to suppress plant genes and enzymes that control the biosynthesis of the most toxic compounds.

75
Johnson EM, Chun YH. IN VITRO DIFFERENTIAL DEVELOPMENTAL TOXICITY OF VITAMIN A CONGENERS. Teratology 1989 Apr;39(4):349-61.

Several forms of vitamin A were tested in the in vitro hydra assay for their developmental toxicity hazard potential and site of action on progressive ontogenesis. Retinol, retinyl acetate, retinaldehyde, all trans retinoic acid, and 13 cis retinoic acid were tested, and each established as being able to perturb development of artificial hydra "embryos: at, or near, adult toxic treatment levels. Consistent with tests of other chemicals, the concentrations needed to produce effects in hydra bore no relation to those needed to produce effects in mammals.

EMBRYOTOXICITY

76

Polley SR, Lloyd PS, Navesey MI. A NOVEL IN-VITRO TECHNIQUE TO DETERMINE EMBRYONIC HEART RATE FROM VIDEO IMAGES. Teratology 1990 Aug;42(2):30A-31A.

Measurement of embryo heart rate can provide an essential insight into cardiac function in response to xenobiotics. Previous instrumental techniques employed to assess heart rate have included: Electrocardiographic measurements, Doppler blood flow measured by probes placed against the myometrium, ultra-sound and the use of laser imaging. Here, a simple, low cost, non-invasive method of monitoring the heart rate of intact day 12 1/2 post coitum rat embryos in-vitro is proposed. This method employs a microscope fitted with a video camera to view embryonic activity on a monitor at a magnification of typically x20. A probe containing a 9mm diameter photodiode is placed over the image of the beating heart. The electrical output from this diode, varying with changes in light intensity, is amplified, filtered and compared against a preset threshold to provide a digital output. The average error of the system has been calculated to be 2%. The investigation utilized a circulating medium, stationary embryo culture system which provided physical and visual accessibility.

77

Hill JA, Polgar K, Harlow BL, Anderson DJ. EVIDENCE OF EMBRYO- AND TROPHOBLAST-TOXIC CELLULAR IMMUNE RESPONSE(S) IN WOMEN WITH RECURRENT SPONTANEOUS ABORTION. Am J Obstet Gynecol 1992;166(4):1044-52.

Study purpose was to determine whether reproductive antigens stimulate lymphocytes and macrophages obtained from women with recurrent abortion who may secrete factors that are toxic to preimplantation embryos or trophoblast cells in vitro. Mononuclear cells were isolated from 30 fertile controls and 300 nonpregnant women being evaluated for recurrent abortion. Supernatants generated from these cells after separate culture with sperm and trophoblast antigen extracts were added to two-cell mouse embryo cultures and trophoblast proliferation assays. Toxicity was assumed when the median percentage of embryos developing to blastocysts or trophoblast proliferation was less than or equal to 50% of control values. Mouse embryo development and/or trophoblast proliferation were significantly inhibited by supernatants from trophoblast and/or sperm antigen-activated peripheral

blood leukocyte cultures from a majority of the 300 women with recurrent abortion but not from 30 women with normal reproductive histories. Embryo-toxic factors were produced by activated leukocyte cultures from 90% of 180 women with a history of recurrent abortion of unexplained etiology, whereas trophoblast-inhibitory factors were detected in 50% of women from the same group. The authors concluded that recurrent abortion in some women is associated with embryo- and/or trophoblast-toxic factor production in response to stimulation by sperm or trophoblast antigens and that the principal factor may involve the 18 kilo-dalton, heat-labile, T-lymphocyte cytokine interferon gamma. This study suggests a new cause of recurrent abortion.

78
Nakaura S, Tanaka S, Usami M, Kawashima K, Hagita K, Kobayashi K, Enomoto M, Takanaka A. IN VITRO TERATOGENICITY TESTING USING THE RAT EMBRYO CULTURE SYSTEM: (4) COMPARATIVE STUDY OF EMBRYOTOXICITY OF CYCLOPHOSPHAMIDE IN VITRO AND IN VIVO. Senten Ijo 1990 Sep;30(3):274.

The in vitro teratogenicity of cyclophosphamide (CP), a teratogen which requires metabolic activation, was compared with its in vivo activity. Rat embryos at day 9.5 of gestation were cultured for 48 hr in rat serum with CP (1.25, 2.5, 5.0 and 10.0 ug/mL) in the presence of metabolic activation system (postmitochondrial fraction of rat liver, NADPH and glucose-6-phosphate). In the in vivo experiment, pregnant rats received intraperitoneal injections of CP (1.25, 2.5, 5.0 and 10.0 mg/kg/day) once daily from days 9 to 11 of gestation, and the embryos were examined at 3 hr after the final injection. CP induced similar growth retardation and the same malformations in both in vitro and in vivo experiments. The results suggest that this in vitro teratogenicity test can estimate the teratogenic potential of chemicals which require metabolic activation.

79
Klug S, Schwabe R, Wildi L, Neubert D. ATTEMPTS TO INTERPRET THE RELATIVE EMBRYOTOXIC POTENCY OF SIX BETA-BLOCKERS. Teratology 1990 Aug;42(2):30A.

Rat embryos (9.5-day-old) were exposed to various concentrations of: acebutolol (AC), atenolol (AT), alprenolol (AL), metoprolol ME), pindolol (PI) and

propanolol (PR) for 48 hours in culture. Concentration-effect-relationships were established for each compound with respect to embryotoxicity in vitro. Although inducing a very similar pattern of abnormalities, the compounds exhibited a wide range of embryotoxic potency. Before attempting to rate the relative embryotoxicity one must consider further properties of these compounds, such as pharmacological efficiency, protein- binding and lipophilic properties. While there is no correlation with either the embryotoxic potency and the pharmacological efficiency or the extent of protein-binding of the compounds, there is good correlation with their lipophilic properties. This may indicate that the transfer to the embryonic tissues is the limiting factor. Consequently, it is essential for the interpretation of the data to measure the embryonic tissue levels of the tested compounds in vitro. An adequate analytical method is now being established in the author's laboratory.

80
Lyng RD. TEST OF SIX CHEMICALS FOR EMBRYOTOXICITY USING FETAL MOUSE SALIVARY GLANDS IN CULTURE. Teratology 1989 Jun;39(6):591-9.

Many new chemicals come into use each year, and the need for rapid and cost-effective methods for testing developmental toxicity is apparent. Establishing reliable in vitro techniques is important to a tiered approach in testing for developmental toxicity. The fetal mouse salivary gland was selected as a possible test system because several interacting developmental processes occur during gland growth, the development of which is quantifiable by counting lobes. For each chemical tested, 20 glands from 13-day embryos were treated in a control media and in three concentrations of the test chemicals. The number of lobes present after 48 hours is dependent on the number of lobes at explantation. Glands with different numbers of lobes at explantation were compared by dividing the number of lobes present after 48 hours by the number present at explantation to determine a growth ratio. Mean growth ratios were used to construct dose-response curves, and from these curves the concentration that reduced growth by 50% (TP50) was determined. Comparisons with in vivo data were made.

81
Peters PW J, Piersma AH. USE OF ECHINOIDS IN IN VITRO EMBRYOTOXICITY AND TERATOGENICITY STUDIES. REPLY TO COMMENTS. Toxicol in Vitro 1992;6(2):179.

The authors are grateful to G. Pagano and N. M. Trieff for expressing their agreement regarding their review on in vitro test systems in teratology. An inherent problem of a review is the limited space available to cover a large and complex field of research. Thus extensive discussion on exposure routes, as well as a more detailed presentation of supporting technical information and a comprehensive bibliography were avoided in favor of general considerations on the application of test systems. Indeed one message of the authors review is that the applicability of a given system should be determined by way of thorough validation. Results of such challenges should ultimately determine the popularity and utility of test systems.

82
Kronauer K, Groth G, Freundt KJ. ZEBRAFISH EMBRYOGENESIS UNDER THE INFLUENCE OF FORMAMIDE DERIVATIVES AND ALKYL AMINES. Naunyn Schmiedebergs Arch Pharmacol 1990;341(Suppl):R21.

Isolated individual fertilized eggs from zebrafish (Brachydanio rerio) developed in vitro can be used to elucidate possible actions of hazardous agents on embryogenesis. This non-mammalian test system is easy to handle and offers opportunity to obtain reproducible results within 4-6 days. In the present study, the following were important indicators of development which were checked at different stages during hatching: epiboly, yolk clot on primitive mouth (stage (st): 16), irregular muscle movement (st: 20), tail bud separation from the yolk (st: 20), heart beats: 200/min (st: 24), hatching of the embryo (st: 25), 34 somites (st: 25), delay of development. Normal development of each parameter (as %) was plotted against the concentrations of each agent applied in the incubation media. Results show that the fish egg model allows investigators to easily differentiate types of embryogenesis lesions caused by chemicals.

83
Shane BS. DEVELOPMENT AND USE OF IN-VITRO AND IN-VIVO ASSAYS TO SCREEN FOR EMBRYOTOXIC AND TERATOGENIC COMPOUNDS IN ANIMALS AND MAN. 203rd ACS (American Chemical Society) National Meeting, San Francisco, California, USA, April 5-10, 1992. Abstr Paper Am Chem Soc 1992; 203(1-3):ENVR290.

No abstract.

84
Flynn TJ, Scialli AR, Gibson RR. CULTURED ORGANOGENESIS-STAGES RAT EMBRYOS AS BIOMARKERS FOR NUTRITIONAL FACTORS IN HUMAN REPRODUCTIVE FAILURE. Teratology 1991 May;43(5):468.

Sera from women with a history of chronic spontaneous abortion adversely affects in vitro development of postimplantation rodent embryos when used as the embryo culture medium (Ferrari et al., Teratology 33:84C, 1986). The present study re-examines the findings of Ferrari et al. in a controlled, double-blind clinical trial. Forty female subjects with a history of chronic reproductive failure were recruited. Headfold stage (GD9) rat embryos were cultured in each subject's serum both with and without a nutrient (amino acids and vitamins) supplement. Of the 11 serum specimens that have undergone preliminary screening, only one allowed normal

growth and development of rat embryos without nutrient supplementaion. For comparison, embryos were cultured in serum specimens obtained from 10 laboratory workers (8 male and 2 female). Ten percent of these embryos had neural tube

defect (NTD) among the 22% of total embryos with defects. These findings suggest that embryotoxic serum factors are more prevalent in women with poor reproductive histories than in a control population, and that in vitro embryotoxicity of some of these sera can be eliminated by supplementation with nutrients.

85
Lyng RD, Scalf R, Monteith D. METABOLIC ACTIVATION IN THE FETAL MOUSE SALIVARY GLAND CULTURE SYSTEM WITH RAT HEPATOCYTES, RAT S-9, AND HUMAN S-9. Teratogenesis Carcinog Mutagen 1991;11(1):31-9.

The usefulness of an in vitro assay for embryotoxicity may depend on the availability of metabolic activation systems that can function in the culture system. The fetal mouse salivary gland has been investigated as an in vitro assay system. To see if glands would grow in the presence of metabolic activators and if glands would react to metabolites known to be embryotoxic, the glands were grown in the presence of cyclophosphamide (CP) and several

activation systems. These included isolated rat hepatocytes, uninduced rat S-9, rat S-9 induced with 3-methylcholanthrene (3-MC), rat S-9 induced with Aroclor 1254, and human S-9. Twenty salivary glands were isolated from 13 day embryos (plug day = 0) and were grown in each treatment for 48 hours. The greatest suppression of salivary gland growth occurred in co-culture with hepatocytes activating CP. The S-9 induced by Aroclor 1254 was nearly as effective as the hepatocytes. The next most effective was a group with similar activity consisting of the uninduced rat S-9 and the three samples of human S-9. The 3-MC-induced S-9 was the least effective in suppressing growth of salivary glands. All the activation systems tested can be used with the salivary gland culture system.

86
Newall DR. STRAIN DIFFERENCE IN THE RESPONSE OF EMBRYONIC CELLS TO TERATOGENS AS DETERMINED IN VITRO USING THE MICROMASS TEST. Teratology 1990 Aug;42(2):28A.

The micromass test is potential as an in vitro screen for teratogens using embryonic cell cultures (Flint & Orton, 1984, Toxicol. Appl. Pharmacol. 76:383-395), and is currently the subject of an international collaborative exercise. As participants in this exercise, we have recently had the opportunity to review some preliminary results from other laboratories. It was interesting to find that there were differences between reported IC50 values for both differentiation and cytotoxicity and our own, for a number of compounds. One possible reason for this was a strain difference in the response of the embryonic cells, since a number of different rat strains had been used. Strain difference in vivo is widely recognized as a major consideration in the design and interpretation of regulatory studies. To determine whether it is of equal importance in in vitro studies, rat IC50 values were determined for the effects of a series of known teratogens on limb-bud and midbrain cell differentiation and survival using tissues from different strains of rat. Results show marked differences between strains particularly for effects on differentiation. For all-transretinoic (TRA), the micromass test is subject to strain variation, although this had no effect on the prediction of teratogenic potential. Results for other teratogens is presented and the origin and importance of strain difference in this test, is discussed.

87
Peters PW, Piersma AH. IN VITRO EMBRYOTOXICITY AND

TERATOGENICITY STUDIES. Toxicology In Vitro 1990; 4(4-5):570-6.

During the past decade many publications have appeared describing test methods for in vitro toxicological research and emphasizing their desirability, appropriations and necessity. One reason is the pressure imposed on regulatory, industrial and academic communities by society to reduce the number of animals used in research and testing strategies. In addition, sophisticated analytical techniques have been developed that allow the measurement of small quantities of biologically important material. Moreover, the present knowledge gained in the area of tissue culture and in vitro embryo culture allows the application of these techniques to more routine studies, as well as studies on mechanisms of action of teratogens in model systems of isolated developmental processes. With respect to reproductive toxicity, embryotoxicity and teratogenicity diverse systems are now available ranging in organizational complexity from bacteria, insects, invertebrates, lower vertebrates, avian embryos and mammalian cells, tissues and organs to whole rodent embryos. This report serves as an introduction to the complex issues raised by the many methods available.

GENETIC TOXICOLOGY
88
Mackay JM, Elliott BM. Series: 'CURRENT ISSUES IN MUTAGENESIS AND CARCINOGENESIS', No. 29: DOSE-RANGING AND DOSE-SETTING FOR IN VIVO GENETIC TOXICOLOGY STUDIES. Mutat Res 1992;271(1):97-9.

In vivo genetic toxicology assays, such as the mouse bone marrow micronucleus test and the rat-liver unscheduled DNA synthesis assay, hold a key position in screening strategies for identifying possible carcinogens, and are often used to overrule clear genotoxic activity seen in vitro. Regulatory guidelines recommended the use of maximum tolerated dose (MTD) or a dose level producing some indication of cytotoxicity at the target organ for the assay. For test materials where relevant toxicity data are available, the dose level scheme is entered at an appropriate dose level and then followed as detailed above until an MTD can be selected for use in the main study. Advantages of the procedure are: (i) it minimizes the use of animals and the severity of toxicity induced by initially carrying out a stepwise dose-ranging procedure with small number of animals; (ii) it does not result in the high levels

of lethality observed during the determination of an median LD, and (iii) the dose level selected is unlikely to cause significant lethality in the main study. The procedure could be used for any in vivo genetic toxicology assay where an MTD is to be determined.

GENOTOXICITY

89
Combes RD. THE IN VIVO RELEVANCE OF IN VITRO GENOTOXICITY ASSAYS INCORPORATING ENZYME ACTIVATION SYSTEMS. Prog Drug Metab 1992;13(0):295-321.

A review with many references. Modification of metabolizing systems, metabolism by indicator cells, and activation by cytochrome P 450 isoenzymes are discussed.

90
Johnson BT. AN EVALUATION OF A GENOTOXICITY ASSAY WITH LIVER S9 FOR ACTIVATION AND LUMINESCENT BACTERIA FOR DETECTION. Environ Toxicol Chem 1992;11(4):473-80.

A new short-term in vitro genotoxicity assay with marine bioluminescent bacteria was evaluated for sensitivity and cost. Known under the trade name of Mutatox, this assay is a simple and rapid screening tool that detects DNA-damaging substances (genotoxins) by measuring light output from an isolated dark mutant strain of the luminescent bacterium Photobacterium phosphoreum. A positive response indicates the ability of the test chemical to restore the luminescent state in the dark mutant strain; the degree of light increase indicates the relative genotoxicity of the sample. In the study, the Mutatox assay with rat hepatic fractions (S9) as an exogenous metabolic activation system detected genotoxic activity with known progenotoxins. Known nongenotoxic controls carbofuran, di-2-ethylhexyl phthalate, malathion, simazine, and permethrin showed no genotoxic responses. The Mutatox assay compared favorably in sensitivity with the Ames test; it was easier and more rapid to perform and cost less. The sensitivity, specificity, and predictive value suggest that the Mutatox assay could have utility as a valuable screening tool to monitor complex environmental samples for genotoxins.

91
Lawrence JN, Benford DJ. DETECTION OF CHEMICAL-INDUCED

UNSCHEDULED DNA SYNTHESIS IN CULTURES OF NORMAL ADULT HUMAN KERATINOCYTES. Sixth International Workshop on In-vitro Toxicology, Domaine de Seillac, France, October 1-6, 1990. Toxicol In Vitro 1991;5(5-6):377-382.

No Abstract.

92
Choy WN, Willhite CC, Henika PR, Omaye S. INCORPORATION OF A MICRONUCLEUS STUDY TO A REPRODUCTIVE TOXICOLOGY AND PHARMACOKINETIC STUDY OF L SELENOMETHIONINE IN LONG-TAILED MACAQUES MACACA-FASCICULARIS. 23rd Annual Scientific Meeting of the Environmental Mutagen Society, Reno/Sparks, Nevada, USA, March 15-19, 1992. Environ Mol Mutagen Suppl 1992;0(20):9.

No abstract.

93
Brusick D. IS THERE SCIENTIFIC JUSTIFICATION FOR CONDUCTING GENETIC RISK ANALYSES FOR USE IN REGULATING CHEMICALS AS HUMAN MUTAGENS? Teratology 1990 Sep;42(3):325.

A series of circumstances has led to proposals that chemicals in high production or with probable high human exposures that express genetic toxicity in vitro or in somatic tissues in vivo should be subjected to genetic risk analysis using the Mouse Specific Locus Test or other mouse germ cell models. Chemicals with high human exposures are often also evaluated for other types of non-reversible toxicity including carcinogenesis and teratogenesis. Evaluation of the existing data base for chemicals subjected to genetic risk assessment has produced results which could be used to argue that adequate cancer and teratology bioassays would provide sufficient information to protect mammalian germ cells (at least males) from genotoxic agents. Data supporting this conclusion will be presented along with limitations of this assumption.

94
Kramers PG, Knaap AG, van der Heijden CA, Taalman RD, Mohn GR. ROLE OF GENOTOXICITY ASSAYS IN THE REGULATION OF CHEMICALS IN THE NETHERLANDS:CONSIDERATIONS AND EXPERIENCES. Mutagenesis 1992;6(6):487-93.(REF. 99)

This paper discusses genotoxicity testing and data interpretation as conducted and applied in The Netherlands in the context of the regulation of

chemicals. Guidelines were first formulated in 1981 and their use evolved in practice, on the basis of increasing experience at the national and international

levels. The distinction between in vitro assays to detect intrinsic genotoxic properties and in vivo assays as a subsequent phase to show the realization of this potential in an intact organism has been a cornerstone of the Dutch approach. Several critical aspects of the use of short-term genotoxicity tests in sequential schemes are discussed. Examples are given as to how short-term tests contributed to the evaluation of the toxicological potential of chemicals in The Netherlands.

95
Gao N, Aidoo A, Heflich RH. ANALYSIS OF RAT LYMPHOCYTE ACTIVATION OF BENZA[A]PYRENE, 2-ACETYLAMINOFLUORENE, AND SEVERAL OF THEIR METABOLITES TO MUTAGENIC AND DNA-DAMAGING SPECIES IN VITRO. Teratogenesis Carcinog Mutagen 1991;11(2):65-76.

Rat lymphocytes are a potentially useful and convenient cell system for monitoring the genotoxic effects of chemicals in vivo, but little is known about the ability of these cells to metabolize promutagens to chemical species that are genotoxic. In this study, Fischer 344 rat lymphocytes were treated in vitro with benzo[a]pyrene (BaP), 2-acetylaminofluorene (2-AAF), and several of their metabolites, and DNA damage was measured using nucleoid sedimentation analysis. Although rat lymphocytes may metabolize certain proximal genotoxic chemicals to DNA-damaging species (e.g., N-hydroxy-2-AAF), the data suggest that in vivo lymphocyte DNA damage is more likely to result from lymphocytes encountering reactive chemical derivatives produced by other cells. It is also clear that differences exist between the ability of human and rat lymphocytes to activate promutagens, and this may impact on the validity of use of the rat model to predict the genotoxicity of chemicals in humans.

96
Howard W, Hoffman S, Kochhar TS. EFFECT OF ARSENIC IN CAUSING CHROMOSOME CHANGES IN CULTURED MAMMALIAN CELLS. 23rd Annual Scientific Meeting of the Environmental Mutagen Society, Reno/Sparks, Nevada, USA, March 15-19, 1992. Environ Mol Mutagen Suppl 1992;0(20):26.

No abstract.

97
Sofuni T, Galloway SM, Ishidate M JR, Shelby MD, Murli H, Thilagar A, Gulati K, Putman DL, Marshall R, Tanaka N. A FURTHER INTERNATIONAL COLLABORATIVE STUDY ON TWO DIFFERENT EXPERIMENTAL SYSTEMS OF IN-VITRO CHROMOSOMAL ABERRATION TESTS. 23RD Annual Scientific Meeting of the Environmental Mutagen Society, Reno/Sparks, Nevada, USA, March 15-19, 1992. Environ Mol Mutagen Suppl 1992;0(20):59.

No abstract.

98
Kirkland DJ. CHROMOSOMAL ABERRATION TESTS IN VITRO: PROBLEMS WITH PROTOCOL DESIGN AND INTERPRETATION OF RESULTS. Mutagenesis 1992; 7(2):95-106.

In vitro chromosomal aberration (CA) tests have come to play a central role in testing for the

mutagenic/carcinogenic potential of chemicals in most countries. Guidelines on the conduct of such assays have therefore been published by a variety of sources, and, if anything, recommended protocols have become even more extensive as time has progressed and revisions have been made. Yet there is very little comparative data from within or between laboratories to form a basis for these recommendations. Claims that certain cell types were more sensitive than others for CA testing has led to comparisons between Chinese hamster ovary (CHO), Chinese hamster lung cells and human lymphocytes, and some examination of critical factors such as exposure periods and sampling times has been undertaken, but more needs to be done. Proposals are made for improved study design, in particular the spacing of doses to permit categorization of chemicals as high toxicity clastogen (HTC) or low toxicity clastogen (LTC). A concept of comparing minimal positive (clastogenic) dose with an arbitrary level of toxicity (e.g. 40-50% inhibitory dose) is introduced to permit this categorization. As an indication of likely in vivo hazard, categorizing a chemical as either HTC or LTC can help with decision making in industry and risk assessment by industrialists and regulators.

99
Whong WZ. DEVELOPMENT OF A LUNG-CELL MODEL FOR STUDYING WORKPLACE GENOTOXICANTS. Govt Reports

Announcements & Index (GRA&I), Issue 04, 1992.

Objectives of the study were to establish in vivo and/or in vitro multiple genetic endpoint assay systems using lung cells of the rat, to compare the sensitivity of rat lung cells to genotoxicants between in vivo and in vitro assay systems, and to evaluate the suitability of the multiple genetic endpoint/lung cell assay system for detecting genotoxicity. Male CD-rats were used in the study. Results indicate that the best enzymatic separation of rat lung cells was treatment of lung which combined trypsin and collagenase or a cold digestion with protease. Primary lung cells can be used for in vivo and in vitro sister chromatid exchange and MN analyses. Both alveolar macrophages and primary lung cells could be used for in vivo and in vitro unscheduled DNA synthesis assays.

HEPATOTOXICITY
100
Cho J-H, Jeong S-H, Jean Y-H, Park J-M, Lee M-H, Lee C-E, Yun H-I. STUDIES ON THE DEVELOPMENT OF METHODS FOR HEPATOTOXICITY TEST. Res Rep Rural Dev Adm (Suweon) 1991;33(3 Vet.):43-54.

To investigate the mouse primary-cultured hepatocytes as a simple and economic model for hepatotoxicity test of chemicals, the characters of carbon tetrachloride (CCl4)-induced hepatotoxicities in primary cultured mouse hepatocytes were compared with those in mice. It was concluded that CCl4-induced hepatotoxicities in mouse primary cultured hepatocytes were almost the same as those in vivo, so this in vitro test using mouse primary cultured hepatocytes may prove to be an economic, feasible and precise model for hepatotoxicity testing.

IMMUNOTOXICITY
101
Wood SC, Karras JG, Holsapple MP. INTEGRATION OF THE HUMAN LYMPHOCYTE INTO IMMUNOTOXICOLOGICAL INVESTIGATIONS. Fundam Appl Toxicol 1992;18(3):450-9.

The xenobiotics acetoxydimethylnitrosamine (ACDMN), acrolein (ACR), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) are potent immunosuppressive agents of the in vitro primary humoral response of murine splenocytes. Focus of these studies was to determined if human lymphocytes could be modulated by direct exposure to these xenobiotics and

therefore be used as in vitro model system for immunotoxicological investigations. Compared was the profile of activity of these xenobiotics on cultured murine splenocytes (SPLC) and human tonsillar lymphocytes (HTL). The studies suggest that HTL can provide a comparable profile of activity as murine SPLC and can therefore be utilized for evaluating the direct immunotoxic potential of certain xenobiotics.

102
Jung M, Agut H, Candotti D, Ingrand D, Katlama C, Huraux JM. SUSCEPTIBILITY OF HIV-1 ISOLATES TO ZIDOVUDINE: CORRELATION BETWEEN WIDELY APPLICABLE CULTURE TEST AND PCR ANALYSIS. J Acquired Immune Defic Syndr 1992;5(4):359-64.

Thirteen isolates of human immunodeficiency virus type 1 (HIV-1) obtained in co-culture with peripheral blood lymphocytes were tested for in vitro susceptibility to zidovudine (ZDV). Seven isolates were obtained from patients who had never been treated with ZDV and six from patients receiving the drug. The seven isolates from untreated patients and four of six from treated patients were susceptible to ZDV. The two isolates from the patients treated for the longest periods were resistant to the drug. The presence of mutations at critical positions of the reverse transcriptase gene was investigated by direct sequencing of polymerase chain reaction (PCR)-amplified DNA and four isolates were found to be mutants. Results suggest that any HIV isolate provided by conventional co-culture could be confidently tested for ZDV susceptibility in order to

study the emergence of resistance during long-term therapy.

103
Smialowicz RJ. IN VITRO LYMPHOCYTE PROLIFERATION ASSAYS: THE MITOGEN-STIMULATED RESPONSE AND THE MIXED LYMPHOCYTE REACTION IN IMMUNOTOXICITY TESTING. Govt Reports Announcements & Index (GRA&I), Issue 06, 1992.

The report describes detailed methodologies for the determination of the in vitro mitogen-stimulated response and one-way mixed lymphocyte reaction of mouse and rat lymphocytes. A list of reagents, supplies and equipment necessary for the successful completion of these assays is provided. Also, a step-by- step description of each of these assays is presented, so

that laboratories unfamiliar with these procedures should be able to perform them. These assays are useful in the identification and characterization of agents capable of altering the immune system and as such are of utility as screening tests for potential immunotoxicants.

104
Luster MI, Portier C, Pait DG, White KL Jr, Gennings C, Munson AE, Rosenthal GJ. RISK ASSESSMENT IN IMMUNOTOXICOLOGY. I. SENSITIVITY AND PREDICTABILITY OF IMMUNE TESTS. Fundam Appl Toxicol 1992;18(2):200-10.

The authors previously reported on the design and content of a screening battery involving a tier approach for detecting potential immunotoxic compounds in mice. This battery has now been utilized in examining a variety of compounds by the NIEHS Immunotoxicology Laboratory, the National Toxicology Program's sponsored laboratories, and by the Cell Biololgy Department at the Chemical Industry Institute of Toxicology. The database generated from these studies, consists of over 50 selected compounds, has been collected and analyzed in an attempt to improve future testing strategies and provide information to aid in quantitative risk assessment for immunotoxicity. Studies presented have established the ability of each of the tests or test combinations in the screening battery to detect immunotoxic compounds. The performance of only 2 or 3 immune tests is sufficient to predict immunotoxic potential of compounds to rodents (>90% concordance). The relationship between immunotoxicity and carcinogenicity, as well as genotoxicity, was also determined. These analyses suggest that potential immunotoxic compounds are likely to be rodent carcinogens although for compounds that are not immunotoxic the carcinogenic status is unclear. There was no relationship observed between immunotoxicity and mutagenicity as determined using in vitro genotoxicity tests. The significance of these observations is discussed.

105
Dearman RJ, Kimber I. IMMUNOTOXICOLOGY AND ALLERGY: OPPORTUNITIES FOR IN VITRO ANALYSIS. Toxicol in Vitro 1991;5(5-6):519-24.

No abstract. (40 REFS)

KIDNEY
106
Walker C, Ginsler J. DEVELOPMENT OF A QUANTITATIVE IN VITRO TRANSFORMATION ASSAY FOR KIDNEY EPITHELIAL CELLS. Carcinogenesis (Eynsham) 1992;13(1):25-32.

A quantitative in vitro transformtion assay has been developed for the first time using primary rat kidney epithelial (RKE) cells. RKE cells were grown in a 50:50 mixture of 3T3 conditioned medium and DF8 medium composed of Ham's F-12/DMEM supplemented with ferrous sulfate, vasopressin, triiodothyronine, insulin, cholesterol, hydrocortisone, transferrin, sodium selenite and 10% fetal bovine serum. Colony forming efficiency of cells plated in this medium was high, ranging from 2.4 to 16%. Normal RKE cells treated with N-methyl-N'-nitro- N-nitrosoguanidine (MNNG) became transformed to a preneoplastic state of enhanced in vitro growth potential and formed large colonies of morphologically altered cells, whereas RKE cells treated with vehicle alone ceased proliferating and/or sloughed off the dish within 4-6 weeks. One of six transformed RKE cell lines injected into nude mice produced adenocarcinomas. This assay may represent an in vitro model for studying mechanisms of chemical transformation of normal kidney epithelial cells and may prove useful as a screen for identifying potential renal carcinogens.

LIVER
107

Lacarelle B, Rajaonarison JF, Gauthier T, Placidi M, Catalin J, Rahamani R. USE OF A HUMAN LIVER MICROSOME BANK IN DRUG GLUCURONIDATION STUDIES. Sixth International Workshop on In-vitro Toxicology, Domaine de Seillac, France, October 1-6, 1990. Toxicol In Vitro 1991;5(5-6):559-562.

No abstract.

MEMBRANES
108
Kessler L, Aprahamian M, Keipes M, Damge C, Pinget M, Poinsot D. DIFFUSION PROPERTIES OF AN ARTIFICIAL MEMBRANE USED FOR LANGERHANS ISLETS ENCAPSULATION: AN IN VITRO TEST. Biomaterials 1992;13(1):44-9.

Glucose and insulin permeability of an artificial

membrane (AN69) used for Langerhans islets encapsulation were investigated. This in vitro test mimicks in vivo conditions and may be of utility to rapidly evaluate the physicochemical properties of a membrane suitable for pancreatic islets encapsulation.

109
Janz S, Shacter E. CYCLODEXTRIN ENCAPSULATION AND DELIVERY OF ALKANES, ESPECIALLY PRISTANE, TO LIVING MAMMALIAN CELLS FOR RISK ASSESSMENT AND PHARMACEUTICAL APPLICATIONS. U. S. Pat. Appl. PATENT NO. 723240 02/15/92 (United States Dept. of Health and Human Services).

A complex of a cyclodextrin and an alkane, alkene, etc. is described. Complexes can be delivered to prokaryotic and eukaryotic cell, tissues, and organs in vitro and in vivo. In this manner, the toxic, genotoxic, and mitogenic effects of these compounds may be determined.

110
Dresser R. STANDARDS FOR ANIMAL RESEARCH: JUSTIFICATION AND ASSESSMENT OF ALTERNATIVES. J Am Vet Med Assoc 1992 Mar 1; 200(5):667-9. (12 REFS)

No abstract.

111
Anderson C. ANIMAL RESEARCH. MILITARY STANDS FIRM ON DISSECTION. [news] Nature 1992 Apr 16;356(6370):554.

No abstract.

112
Hoffman AD, Bertelsen SL, Gargas ML. AN IN VITRO GAS EQUILIBRATION METHOD FOR DETERMINATION OF CHEMICAL PARTITION COEFFICIENTS IN FISH. Comp Biochem Physiol a Comp Physiol 1992;101(1):47-52.

A gas equilibration method for the determination of in vitro chemical partition coefficients in mammals was adapted for use with fish. In vitro blood; water and tissue: blood partition coefficients were determined for three chlorinated ethanes in rainbow trout (Oncorhynchus mykiss). In vitro partition coefficients accurately predicted chemical concentrations in tissues

of exposed trout.

MUTAGENESIS

113
Clonfero E, Saia B. THE AMES TEST IN ENVIRONMENTAL AND OCCUPATIONAL HEALTH. Medicina del Lavoro Jan.-Feb. 1990:81(1):3-10. (78 REFS)

A review of the use of the gene mutation test using Salmonella typhimurium (Ames test) as applied to problems in environmental and occupational health. This test, originally intended as a test predictive of the carcinogenicity of chemical substances, has been applied in the in vitro screening of complex mixtures of substances present in the environment and as means to monitor high risk populations. Data are reported on the main environmental exposures that were positive in the Ames test and that this biological assay has contributed to the identification of new classes of genotoxic compounds. The Ames test performed on extracts of human urine was used to study exposure to carcinogenic substances in the working environment. Many occupational exposures can cause an increase in mutagenic activity in the urine of exposed subjects. It is recommended that the use of the urinary mutagenesis test be restricted to group studies and that confounding factors (e.g. smoking, diet) be carefully checked.

114
Tummey AC, Mckee RH, Przygoda RT. EVALUATION OF IN-VIVO TUMOR PROMOTERS IN A BATTERY OF IN-VITRO ASSAYS. 23RD Annual Scientific Meeting of the Environmental Mutagen Society, Reno/Sparks, Nevada, USA, March 15-19, 1992. Environ Mol Mutagen Suppl 1992;0(20):66.

No abstract.

115
Kumaroo PV, Thilager A, Facundo N, Mcmurrin WM, Anderson BE, Zeiger E. IN-VITRO CHROMOSOME ABERRATION AND SISTER CHROMATID EXCHANGE TESTS OF 35 CHEMICALS IN CHINESE HAMSTER OVARY CELLS. 23RD Annual Scientific Meeting of the Environmental Mutagen Society, Reno/Sparks, Nevada, USA, March 15-19, 1992. Environ Mol Mutagen Suppl 1992;0(20):32.

No abstract.

116
Johnsrud K, McDermott M, Thilager A. SIMULTANEOUS IN-SITU EVALUATION OF CHEMICALLY INDUCED POLYPLOIDY AND MICRONUCLEI IN CHINESE HAMSTER OVARY CELLS. 23RD Annual Scientific Meeting of the Environmental Mutagen Society, Reno/Sparks, Nevada, USA, March 15-19, 1992. Environ Mol Mutagen Suppl 1992;0(20):28.

No abstract.

117
Gu Z-W, Zhong B-Z, Liu Y-Q, Ong T, Whong W-Z. INDUCTION OF MICRONUCLEI SISTER CHROMATID EXCHANGES AND GENE MUTATIONS IN CHINESE HAMSTER V79 AND G12 CELLS BY DIESEL EMISSION PARTICULATES-STANDARD REFERENCE MATERIAL 1650. 23rd Annual Scientific Meeting of the Environmental Mutagen Society, Reno/Sparks, Nevada, USA, March 15-19, 1992. Environ Mol Mutagen Suppl 1992;0(20):22.

No abstract.

118
Gollapudi BB, Mcclintock ML, Zempel JA, Sinha AK. METHODS FOR THE CONDUCT OF IN VITRO RAT HEPATOCYTE UNSCHEDULED DNA SYNTHESIS UDS ASSAY WITH GASEOUS AND VOLATILE TEST MATERIALS. 23rd Annual Scientific Meeting of the Environmental Mutagen Society, Reno/Sparks, Nevada, USA, March 15-19, 1992. Environ Mol Mutagen Suppl 1992;0(20):20.

No abstract.

119
Coelho M C LS, Coimbrao CA, Valent GU, Sato M IZ, Sanchez PS, Targa HJ. MUTAGENICITY EVALUATION OF INDUSTRIAL EFFLUENTS BY AMES ASSAY. 23rd Annual Scientific Meeting of the Environmental Mutagen Society, Reno/Sparks, Nevada, USA, March 15-19, 1992. Environ Mol Mutagen Suppl 1992;0(20):11.

No abstract.

120
Clark LS, Sullivan LM, Larsson CJ, Falta MT, Nicklas JA, O'Neill JP, Huberman E, Collard FR, Voelz GL, Albertini

RJ. MUTANT FREQUENCIES OF PLUTONIUM WORKERS DETERMINED BY HPRT CLONING ASSAY. 23rd Annual Scientific Meeting of the Environmental Mutagen Society, Reno/Sparks, Nevada, USA, March 15-19, 1992. Environ Mol Mutagen Suppl 1992;0(20):10.

No abstract.

121
Christie NT, Tummolo DM, Lee YW, Pons C. THE MUTATIONAL RESPONSE OF G12 CELLS TO NICKEL COMPOUNDS. 23rd Annual Scientific Meeting of the Environmental Mutagen Society, Reno/Sparks, Nevada, USA, March 15-19, 1992. Environ Mol Mutagen Suppl 1992;0(20):10.

No abstract.

122
Ruediger HW. CLINICAL, GENETIC AND REGULATORY CONSEQUENCES OF EXPOSURE TO MUTAGENS. Ann Genet 1992; 34(3-4):173-178.

No single agent is known to cause an increase of genetic disorders in humans. Studies on large numbers of children born to parents after exposure to ionizing radiation or DNA alkylating agents failed to detect significant genetic consequences. This is in contrast to effects observed in human somatic cells or germ cells, and to various investigations with laboratory animals. A definitive explanation of these discrepancies does not exist. There are obvious incongruencies between the potency of an agent to cause mutations at all, and to induce genetic effects in offspring. It is emphasized that a regulatory classification of agents according to a potential genetic hazard in man must not solely be based on in vitro mutagenicity data.

123
Bean CL, Johnson TE, Galloway SM. IS A LATER HARVEST TIME NEEDED TO DETECT CLASTOGENS IN THE CHINESE HAMSTER OVARY CELL CHROMOSOME ABERRATION ASSAY? 23rd Annual Scientific Meeting of the Enviromnental Mutagen Society, Reno/Sparks, Nevada, USA, March 15-19, 1992. Environ Mol Mutagen Suppl 1992;0(20):5.

No abstract.

124
Campbell JA, Allen JS. MUTAGENICITY OF DISPERSE DYES IN AMES SALMONELLA-TYPHIMURIUM AND MAMMALIAN MICROSOME REVERSE MUTATION ASSAY. 23rd Annual Scientific Meeting of the Environmental Mutagen Society, Reno/Sparks, Nevada, USA, March 15-19, 1992. Environ Mol Mutagen Suppl 1992; 0(20):8.

No abstract.

MUTAGENICITY
125
Ritenour ER, Braaton M, Harrison GH, Ueno A, Gadd M, Manco-Johnson M, Parker R, Shih S, Waldren CA. ABSENCE OF MUTAGENIC EFFECTS OF CONTINUOUS AND PULSED ULTRASOUND IN CULTURED (AL) HUMAN-HAMSTER HYBRID CELLS. Ultrasound Med Biol 1991;17(9):921-30.

Mutagenic effects of continuous and pulsed ultrasound were looked at for use in an in vitro assay system (the AL hybrid) that is up to 100 times more sensitive for mutagens such as x-rays and neutrons than the assays used previously to evaluate ultrasound. Cells in suspension in rotated plastic test tubes were insonated with continuous wave ultrasound at 1 MHz, ISPTP = 0.62-40 W/cm2 for 0-40 min. Cells attached in the central region of culture flasks received pulsed exposures at fc = 2.5 MHz, PRF = 1 kHz, 2 and 8 cycles per pulse, with p- = 1.2 MPa (ISPTA = 31-180 mW/cm2) for 0-30 min. Exposures at these levels were cytotoxic (the plating efficiency was decreased to approximately 65% by the highest doses), induction of mutation, if any occurred, was less than would be expected in this test system from 10-30 cGy of x-ray.

126
Wyle-Gyurech GG, Reinhardt CA. DIFFERENTIATION OF EMBRYONIC CHICK BRAIN CELLS IN MONOLAYER AND REAGGREGATE CULTURES A POTENTIAL MODEL IN-VITRO FOR NEUROTOXICITY. Sixth International Workshop on In-vitro Toxiclolgy, Domaine de Saillac. France, October 1-6, 1990. Toxicol in Vitro 1991;5(5-6):419-426.

No abstract.

127
Fountain SB, Ting Y-L T, Teyler TJ. THE IN VITRO HIPPOCAMPAL SLICE PREPARATION AS A SCREEN FOR NEUROTOXICITY. Toxicol In Vitro 1992;6(1):77-88.

One of the current goals of neurotoxicology research is to develop methods of assessing the neurotoxicity potential of chemical agents by means that are sensitive, rapid and economical. Although no single method is likely to fulfill the role of a general toxicity screen for all organ systems, in vitro brain-slice methods may hold the key to increased sensitivity in screening within the more restricted domain of central nervous system toxicity. The hippocampal brain-slice preparation is well suited for screening purposes because the neurophysiology of the hippocampal slice is understood and generally matches what is known about the intact hippocampus. The in vitro hippocampal slice preparation as a screen for neurotoxicity offers the advantages of in vitro methods and may offer guarded, but relatively direct extrapolation to dysfunction of learning, memory and other behavioural processes.

128
Halks-Miller M, Fedor V, Tyson CA. OVERVIEW OF APPROACHES TO IN-VITRO NEUROTOXICITY TESTING. J Am Coll Toxicol 1991; 10(6):727-736.

No abstract.

129
Sawyer TW, Weiss MT, D'Agostino PA, Provost LR, Hancock JR. BIOASSAY OF ORGANOPHOSPHATE NERVE AGENTS IN SOIL USING NEURONAL TISSUE CULTURES. J Appl Toxicol 1992; 12(1):1-6.

A soil sample originating from an area of suspected chemical warfare activity was subjected to chemical analysis and bioassay. Sarin and several related compounds were confirmed in the soil by capillary column gas chromatography-mass spectrometry (GC-MS). The chemical results were compared to those obtained by bioassay in primary cultures of chick embryo forebrain neurons. By comparing the sample's anticholinesterase activity against those of purified standards in chick embryo neuron cultures, a reasonable agreement was found between the chemical and bioassay semi-quantitative estimates of sarin content in the soil extract. The in vitro system appears to offer a sensitive technique for the estimation of sarin remaining bound to the soil following solvent extraction as well as for an assessment of the potential toxicity of

the contaminated soil in vivo.

130
Reinhardt CA, Bienz A, Romano-Diethelm T, Wyle-Gyurech GG. IN VITRO DIFFERENTIATION OF EMBRYONIC CHICK BRAIN CELLS: DEVELOPMENT OF A NEUROTERATOLOGY TEST SYSTEM. Experientia 1991 Feb;47:A5.

In order to develop a model for potential neurotoxicity and teratogenicity, chick brain cells (embryonic day 7, ED7) were mechanically dissociated and cultured for up to several months. Differentiation of nerve and glial cells (in petridishes, monolayers; and suspension cultures as reaggregates) were monitored with monoclonal antibodies against 68kD neurofilament protein (anti-NF) glial fibrillary acidic protein (anti-GFAP) and tyrosine hydroxylase as well as by monitoring the protein synthesis pattern by 2D-gel electrophoresis. Anti-NF stains neurons in vitro as they differentiate morphologically. In reaggregates a stable differentiation of nerve cells could be observed by intensive anti-NF staining for as long as 3 wk in culture. 5 wk old cultures still showed substantial staining. The expression of NF in nerve cells is proposed to be used as a sensitive cytotoxicity end-point whereas GFAP expression can serve as an endpoint for monitoring differentiation of neural tissue (Wyle & Reinhardt, Toxicol. In Vitro, in press). Monitoring of functional parameters of the dopaminergic system (such as activity of tyrosine hydroxylase) and other endpoints (protein metabolism, cytotoxicity) can be combined to evaluate the in vitro toxic potential of known neuroteratogens such as psychoactive drugs and analgetica.

131
St John PA, Stephens SL. DEVELOPMENT OF SUBSTANCE P RECEPTORS ON RAT MOTONEURONS IN VITRO. Dev Biol 1992;151(1):154-65.

Experiments were performed to examine the influence of interneuronal interactions on the expression of neurotransmitter receptors by developing mammalian central nervous system neurons. Receptors for the neuropeptide, substance P (SP), were assayed on embryonic rat motorneurons and other spinal cord neurons developing in vitro by the binding of 125I-SP to live neurons. Scatchard analysis showed the presence of high-affinity binding sites, and binding

competition assays using SP, neurokinin A, or neurokinin B indicated that the high-affinity 125I-SP binding sites on these neurons were type NK1 tachykinin receptors, or SP receptors (SPRs). Neurons in the spinal cords of rats at Embryonic Day 14 displayed no SPRs. Results indicate that rat spinal motoneurons can express SPRs early in their development, and further suggest that the initial expression of SPRs by developing motoneurons does not require interaction with other neurons.

OCULAR TOXICITY

132
Shaw AJ, Balls M, Clothier RH, Bateman ND. PREDICTING OCULAR IRRITANCY AND RECOVERY FROM INJURY USING MADIN-DARBY CANINE KIDNEY CELLS. Toxicol in Vitro 1991; 5(5-6):569-71.

Two promising cell culture assays, using Madin-Darby canine kidney cells, for predicting eye irritancy, the fluorescein leakage assay and the Neutral Red release assay, have been adapted to try and assess the ability of damaged cells to recover from chemical-induced injury. The fluorescein leakage and Neutral Red release protocols are similar but measure injurious effects on different parts of the cells, namely the tight junctions and the cell membrane, respiration. Both endpoints have previously given equivilants rankings of chemicals in order of their eye irritancy potential. Sixteen compounds of varying irritancy potential and chemical nature were tested using the two assays. Comparing the in vitro results with in vivo data suggests that the fluorescein leakage assay, in its current format, does not predict the likely recovery rate of ocular tissue after chemical damage.

133
Mueller-Decker K, Fuerstenberger G, Vogt I, Marks F. DEVELOPMENT OF AN IN-VITRO IRRITANCY TEST AS AN ALTERNATIVE TO THE ANIMAL DRAIZE TEST. Third Winter Meeting of the German Society of Pharmacology and Toxicology, Hannover, Germany, December 4-6, 1991. Naunyn-Schmiedeberg's Arch Pharmacol 1991;344(Suppl. 2):R122.

No abstract.

134

Feder PI, Lordo RA, Dipasquale LC, Bagley DM, Chudkowski M, Demetrulias JL, Hintze KL, Marenus KD, Pape W JW, et al. THE CTFA EVALUATION OF ALTERNATIVES PROGRAM: AN EVALUATION OF IN VITRO ALTERNATIVES TO THE DRAIZE PRIMARY EYE IRRITATION TEST (PHASE I) HYDRO-ALCOHOLIC FORMULATIONS: (PART 1). STATISTICAL METHODS. In Vitro Toxicol 1991;4(4):231-246.

The CTFA Evaluation of Alternatives Program is a preliminary evaluation of the relationship between Draize ocular safety test data and comparable data from a selected in vitro tests. The Program is not a validation exercise, but rather provides information regarding the relative importance of a series of in vitro tests with respect to a representative set of hydroalcoholic formulations. The data consist of the results from 25 in vitro assays and from the Draize test. Results of each assay are represented by a profile of scores, one for each test formulation. Statistical methods were developed and applied to compare the results from the 25 in vitro assays among one another and the results from selected in vitro assays with those from the Draize test. A number of the statistical design issues pertaining to the Program are described, as are the statistical procedures used for data analysis.

135
Xu G-T, Zigler J S JR, Lou MF. ESTABLISHMENT OF A NAPHTHALENE CATARACT MODEL IN VITRO. Exp Eye Res 1992;54(1):73-82.

In the past, almost all studies on naphthalene cataract were based on in vivo experiments. Such studies are laborious and time- consuming and are complicated by systemic toxicity arising from the metabolites of naphthalene. In order to study the direct effects of naphthalene metabolites on the lens, in vitro 'naphthalene cataract' model system was established by exposing rat lens to naphthalene dihydrodiol. Several biochemical parameters including the glutathione level, protein mixed disulfides, protein patterns on SDS-gels, active transport, Na+/K+-ATPase activities and measurement of naphthalene metabolites in the cultured lenses. The results showed that both morphological and biochemical changes were similar to those observed in lenses of rats fed naphthalene. The model system can be used as a new tool to investigate the mechanism of naphthalene cataract formation. Naphthalene metabolites such as 1-naphthol, 2-naphthol,

1,2-dihydroxynaphthalene and 1,2-naphthoquinone were studied in vitro and results showed that the effects of these naphthalene metabolites were very different from those observed in naphthalene cataracts in vivo.

136
Braa SS, Triglia D. PREDICTING OCULAR IRRITATION USING 3-DIMENSIONAL HUMAN FIBROBLAST CULTURES. Cosmet Toilet 1991 Dec;106(Dec):55-58,60.(15 REFS)

The use of a novel, standardized, 3-dimensional human skin substrate in the ocular safety assessment of 15 shampoos using 4 different assay systems with mechanistically distinct endpoints is described and compared to in vivo rabbit Draize unwashed eye scores.

137
Blein O, Adolphe M, Lakhdar B, Cambar J, Gubanski G, Castelli D, Contie C, Hubert F, Latrille F, et al. CORRELATION AND VALIDATION OF ALTERNATIVE METHODS TO THE DRAIZE EYE IRRITATION TEST OPAL PROJECT. Sixth International Workshop on In-vitro Toxicology, Domaine de Seillac, France, October 1-6, 1990. Toxicol in Vitro 1991;5(5-6):555-558.

No abstract.

138
Spielman H, Gerner I, Kalweit S, Moog R, Wirnsberger T, Krauser K, Kreiling R, Kreuzer H, Luepke N-P, et al. INTERLABORATORY ASSESSMENT OF ALTERNATIVES TO THE DRAIZE EYE IRRITATION TEST IN GERMANY. Sixth Interantional Workshop on In-vitro Toxiclolgy, Domaine de Saillac. France, October 1-6, 1990. Toxicol in Vitro 1991;5(5-6):539-542.

No abstract.

ORGAN CULTURE
139
Lyng RD, Scalf R, Monteith D. USING RAT HEPATOCYTES, UNINDUCED AND INDUCED RAT S-9, AND HUMAN S-9 FOR METABOLIC ACTIVATION IN THE FETAL MOUSE SALIVARY GLAND CULTURE SYSTEM. Teratology 1990 May;41(5):575-6.

The usefulness of an in vitro assay for embryotoxicity may depend on the availability of metabolic activation

systems that can function in the culture system. The fetal mouse salivary gland has been investigated as an in vitro assay system. To see if the glands would grow in the presence of metabolic activators and if they would react to metabolites known to be embryotoxic, the glands were grown in the presence of cyclophosphamide (CP) and several activation systems, which included: isolated rat hepatocytes, uninduced rat S-9, rat S-9 induced with methylcholanthrene, rat S-9 induced with arochlor 1254, and human S-9. Twenty salivary glands were isolated from 13 day embryos (plug day = 0) and were grown in each treatment media for 48 hours. All of the activation systems tested can be used with the salivary glands in culture conditions for embryotoxicity testing.

140
Bucklaw AR, Abbott BD. A NEW PROCEDURE FOR SERUM-FREE PALATAL ORGAN CULTURE. Teratology May 1991;43(5):461.

In developmental toxicology it is desirable to have serum-free in vitro methods. A method developed by Shiota (Acta Anat, 1990), allows fusion of mouse palates in serum-free modified BGJb medium. To give optimal developmental conditions for several species, we have made modifications including more frequent media changes and use of disposable flasks. In this study, tissue obtained from CD-1 and C57BL6N mice, F344 rats, and human palates developmed normally. In 25 experiments 186 CD-1 embryos were examined and 6.99% failed to fuse. Similar results were obtained with C57BL/6N mice (6.25%). In 7 experiments with 39 F344 rat embryos, only 18% failed to fuse. Three human carniofacial tissues aged GD 52-56, 53-56 and 53-59 all fused in culture. Proliferation and differention were normal in culture. This method accurately simulates in vivo development and will be a valuable tool in developmental toxicology studies.

ORGAN TOXICITY
141
Fisher R, Hanzlik RP, Gandolfi AJ, Brendel K. TOXICITY OF ORTHO-SUBSTITUTED BROMOBENZENES IN RAT LIVER SLICES: A COMPARISON TO ISOLATED HEPATOCYTES AND THE WHOLE ANIMAL. In Vitro Toxicol 1991;4(3):173-86.

An in vitro mammalian system which can mimic toxicities seen in the intact animal could have an impact on the overall use of animals in research. This study focused on the relative hepatotoxicity of bromobenzene and five

of its ortho-substituted derivatives; ortho-bromobenzonitrile (BBN), ortho-dibromobenzene (DBB), o-bromoanisole (BA), ortho- bromotoluene (BT), and ortho- bromobenzomethyltrifluoride (BBT). These compounds were tested in phenobarbital-induced Sprague-Dawley rats in vivo and in vitro using liver slices. Precision-cut liver slices apparently may be more representative of observed in vivo toxicities than the isolated hepatocyte.

PULMONARY TOXICOLOGY

142

Costa KA, Cerreta JM. LUNG TOXICANTS BLEOMYCIN AND AMIODARONE ALTER CROSSLINKED ELASTIN LEVELS IN AN IN-VITRO MODEL. 1992 Meeting of the Federation of American Societies for Experimental Biology (FASEB), Part I, Anaheim, California, USA, April 5-9, 1992. FASEB (Fed Am Soc Exp Biol) J 1992;6(4):A1065.

No abstract.

REPRODUCTIVE TOXICITY

143

van Aerts L, Piersma AH, Verhoef A, Peters PW, Copius, Peereboom-Stegeman JH. BIOACTIVATION OF CYCLOPHOSPHAMIDE IN AN IN VITRO POSTIMPLANTATION RAT-EMBRYO/HEPATOCYTES CO-CULTURE, USING MATERNAL HEPATOCYTES IN SUSPENSION. Reprod Toxicol 1991;5(3):270.

The in vitro postimplantation rodent embryo culture is widely used as a tool in teratogenicity studies. Since bioactivation of pro-teratogens occurs largely in the maternal compartment, investigated was whether it was possible to co-culture maternal hepatocytes without altering the standardized procedures for in vitro postimplantation embryo culture. Results show that bioactivation of a pro-teratogen in an in vitro postimplantation rat-embryo culture can be achieved by co-culturing maternal hepatocytes in suspension.

144

Mohr KL, Working PK. AN IN VITRO TECHNIQUE TO DETECT DOMINANT LETHAL MUTATIONS INDUCED IN MOUSE OOCYTES BY ETHYL METHANESULPHONATE EXPOSURE IN VIVO. Toxicology In Vitro 1990;4(2):115-21.

Administration of chemical mutagens to the female rodent can induce dominant lethal mutations in oocytes

and affect embryo development after fertilization. Traditional in vivo dominant lethal assays cannot separate specific genotoxic effects on the embryo from generalized cytotoxic effects. Embryo culture was used, after in vivo exposure of oocytes, to separate the genotoxic effects of a chemical on oocytes from effects due to maternal toxicity. The in vitro test can be used to study the effects of chemicals on all stages of zygote development thereby separating induced genotoxic effects from the possible effects of maternal toxicity on zygote development.

145
Greenwald GS. POSSIBLE ANIMAL MODELS OF FOLLICULAR DEVELOPMENT RELEVANT TO REPRODUCTIVE TOXICOLOGY. Reprod Toxicol 1987;1(1):55-9.

Models of follicular development in rodents that may be applicable to reproductive toxicology were considered. The value of evaluating changes in follicular numbers during the estrous cycle is stressed. Most of the methods described involve in vivo manipulations. However, the use of enzymes to dissociate intact follicles from the ovary and their subsequent in vitro development in the presence or absence of xenobiotics offers an alternative, attractive approach.

146
Baeder C, Daston G, Hellwig J, Schmid B, Schon H, Bontinck WJ. ASSESSMENT OF ALTERNATIVE APPROACHES IN REPRODUCTIVE TOXICOLOGY. Teratology Sept 1990;42(3):323.

Much progress has been made in the development of in vivo and in vitro techniques to assess the reproductive toxicity of chemicals. This paper reviews the present position of alternatives to the conventional techniques and assesses their relevance and validity. Most new tests detect teratogenic activity rather than other embryotoxic effects. At present no alternative test can totally replace the existing reproductive toxicity tests which use live animals. Application of the results from the alternative test approaches to human hazard assessment is also discussed. Without additional knowledge from standard in vivo studies, alternative in vivo or in vitro tests are generally not suitable for defining abnormalities which may occur in human reproduction. Information used to judge the probability of chemicals affecting human reproduction can come only from conventional experimental reproductive toxicity studies or from observations in humans.

SKIN

147

Cohen C, Dossou G, Rougier A, Rouget R. MEASUREMENT OF INFLAMMATORY MEDIATORS PRODUCED BY HUMAN KERATINOCYTES IN-VITRO A PREDICTIVE ASSESSMENT OF CUTANEOUS IRRITATION. Sixth International Workshop on In-vitro Toxiclolgy, Domaine de Saillac. France, October 1-6, 1990. Toxicol in Vitro 1991;5(5-6):407-410.

No abstract.

148

Osborne R, Perkins MA. IN-VITRO SKIN IRRITATION TESTING WITH HUMAN SKIN CELL CULTURES. Sixth International Workshop on In-vitro Toxicology, Domaine de Seillac, France, October 1-6, 1990. Toxicol in Vitro 1991; 5(5-6):563-568.

No abstract.

149

Burnham K, Rahman M. EFFECTS OF PETROCHEMICALS AND ULTRAVIOLET RADIATION ON EPIDERMAL IA EXPRESSION IN VITRO. J Toxicol Environ Health 1992;35(3):175-185.

Previously demonstrated was that combined treatment of mice with crude oil and longwave ultraviolet radiation (UVA) led to the depletion of IA-positive cells from the epidermis. Developed in the present study, was an in vitro screening assay for combined effects of purified petrochemicals and UVA on epidermal IA and Thy-1 expression. This method involves removal of skin from donor mice prior to treatment with chemicals and UVA (20,000 J/m2), followed by in vitro culture and subsequent immunoperoxidase staining. The in vitro assay developed for this study should prove valuable for the screening of a wide variety of chemicals for contact photosensitizing activity.

150

Ponec M. RECONSTRUCTION OF HUMAN EPIDERMIS ON DE-EPIDERMIZED DERMIS EXPRESSION OF DIFFERENTIATION-SPECIFIC PROTEIN MARKERS AND LIPID COMPOSITION. Sixth International Workshop on In-vitro Toxicology, Domaine de Seillac, France, October 1-6, 1990. Toxicol In Vitro 1991; 5(5-6):597-606.

No Abstract.

151
Bruner LH. ALTERNATIVES TO THE USE OF ANIMALS IN HOUSEHOLD PRODUCT AND COSMETIC TESTING. J Am Vet Med Assoc 1992 Mar 1;200(5):669-73. (24 REFS)

No abstract.

152
Zhong JP, Zhou RR, Chen GS, Wang Y, Wang JG. INFLUENCE OF VEHICLES ON HUMAN SKIN PERMEATION OF NORGESTREL AND LEVONORGESTREL IN VITRO. Yaoxue Xuebao 1991;26(12):933-7.

Skin permeation cell methods were constructed for investigating the influence of various vehicles on the transdermal absorption of norgestrel (NG) and levonorgestrel (LNG). All of the vehicles were saturated with NG or LNG at 37 degrees, so variations in permeation were mainly attributable to the vehicle effects. The transdermal fluxes of NG and LNG were increased about 5-6-fold, using an optimal concentration range of aqueuus ethanol as a vehicle relative to that when normal saline was used as a vehicle. The NG permeation rate was about 1- to 2-fold greater than that of LNG in the above-mentioned saline, aqueous ethanol and neat ethanol. Oleic acid (OA) and aqueuus ethanol both enhanced the permeation of LNG through the skin of human cadavers.

153
Charaf UK, Hart GL. PHOSPHOLIPID LIPOSOMES/SURFACTANT INTERACTIONS AS PREDICTORS OF SKIN IRRITATION. J Soc Cosmet Chem 1991; 42(Mar-Apr):71-85. (29 REFS)

The use of large unilamellar liposomes is described as a model membrane system to study surfactant-skin interactions. The relative tendency of surfactants or surfactant blends to form mixed micelles with liposome membrane components determines the aggressivity factor believed to be related to in vivo surfactant irritation responses. Single surfactants and surfactant mixtures were investigated and their behavior toward liposomal membranes used to establish a mathematical index of surfactant aggressivity. A statistically significant rank correlation was established between this index and in vivo scores for the anionic surfactants and blends

tested, as well as for some mixed blends, but not with nonionics. Advantages of this method over traditional in vivo tests are described.

154
Kubota K, Maibach HI. ESTIMATION OF THE PERMEABILITY COEFFICIENT FROM A FINITE DOSE, IN VITRO PERCUTANEOUS DRUG PERMEATION STUDY. J Pharm Sci 1991; 80(Oct):1001-1002. (4 REFS)

Equations for the estimation of the permeability coefficient of a drug from experimental data using in vitro tests on one skin piece are proposed.

155
Iwasa A, Narui T, Murata Y, Kaneko T, Asaoka T, Imamori K, Nagai T. CORRELATIONSHIP BETWEEN IN VITRO AND IN VIVO PERCUTANEOUS ABSORPTION OF NETICONAZOLE HYDROCHLORIDE FROM OINTMENT AND THERAPEUTIC ACTIVITY OF NETICONAZOLE HYDROCHLORIDE OINTMENTS AGAINST EXPERIMENTAL CUTANEOUS TRICHOPHYTON MENTAGROPHYTES INFECTION. Yakuzaigaku 1991;51(4):235-40.

The correlation among evaluation methods for the antimycotic activity of neticonazole-HCl (I) ointments using various oily bases were investigated. In vitro percutaneous absorption method using excised hairless mouse skin, in vivo percutaneous absorption method with guinea-pig and for the therapeutic activity on experimental cutaneous T. mentagrophytes infection in guinea-pig were employed. There was a good correlation between the adsorbed amount of the drug in the skin obtained by in vitro and in vivo experiments. Also in vitro and in vivo results correlated with the effect on experimental dermatophytosis. These facts suggested that in vitro experimentation using hairless mouse skin and in vivo experimentation using guinea-pigs were practical methods for screening antimycotic ointments prior further evaluation of the therapeutic activity on experimental dermatophytosis.

156
Morelli JG, Kincannon J, Yohn JJ, Zekman T, Weston WL, Norris DA. LEUKOTRIENE C4 AND TGF-ALPHA ARE STIMULATORS OF HUMAN MELANOCYTE MIGRATION IN VITRO. J Invest Dermatol 1992;98(3):290-5.

Human vitiligo is a disease of melanocyte destruction

that leads to areas of depigmentation in the skin. Immune cytokines and inflammatory mediators may be released as a result of photochemotherapy of vitiligo, and act as signals for melanocyte migration. In an in vitro assay that quantitates the movement of individual cultured melanocytes over a 72-hour period and which uses time-lapse photography, both LTC4 and transforming growth factor-alpha (TGF-alpha) were shown to be stimulators of melanocyte migration in vitro. The LTC4 effect was greater and lasted for the entire 72-hour exptimental period, whereas the TGF-alpha effect was significant only during the 1st 24 hours of the experiment.

157
Herzinger T, Korting HC. IN VITRO METHODS FOR THE EVALUATION OF THE SKIN IRRITANCY OF CHEMICALS, ESPECIALLY SURFACTANTS. Dermatosen Beruf Umwelt 1991;39(4):117-23. (66 REFS)

A review of the title methods using isolated skin, fertilized chicken eggs, or tissue culture systems.

158
Maier K, Schmitt-Landgraf R, Siegemund B. DEVELOPMENT OF AN IN VITRO TEST SYSTEM WITH HUMAN SKIN CELLS FOR EVALUATION OF PHOTOTOXICITY. Toxicol in Vitro 1991;5(5-6):457-61.

An in vitro system using human skin is described for evaluation of photosensitizers and phototoxicity, as well as ultraviolet radiation effects.

TERATOGENESIS
159
Bacon WJ, Duffy PA, Jones K. STUDIES ON VARIABILITY OF THE MICROMASS TERATOGEN TEST. Toxicol In Vitro 1990;4(4-5):577-81.

The micromass assay is an in vitro test be used to predict the teratogenic potential of compounds being developed in the pharmaceutical and chemical industries. The assay is currently undergoing validation by an interlaboratory blind trial at several national and international institutions. The present paper investigates areas of the assay system where variability in technique may influence the reproducibility of the test results. Control of sources

of variability in the micromass assay increased the reproducibility of the results.

160
Combes RD, Innes D. PRELIMINARY STUDIES ON THE USE OF THE IN VITRO RAT MICROMASS ASSAY-RESULTS WITH SOME MODEL TERATOGENS. Toxicologist 1990 Feb;10(1):125.

Cells from rat embryo mid-brain (CNS) and limb buds (LB) were cultured for 5 days to allow differentiation into neurons and chondrocytes, respectively. Inhibition of the differentiation of these into darkly staining foci was scored as an indicator of teratogenicity. Cytotoxicity was assessed after staining with neutral red. Studied was: (a) the ability of the assay to distinguish between teratogens and non-teratogens; (b) variability between experiments: and (c) the impact of cytotoxicity on data interpretation.

161
Flamme I, Albach K, Muller S, Christ B, Jacob HJ. TWO-PHASE IN VITRO CULTURE OF EXPLANTED CHICK EMBRYOS. Anat Rec 1991 Mar;229(3):427-33.

The present study describes a method of culturing chick embryos together with their surrounding area vasculosa on two different culture media in succession. The average length of survival in vitro was found to be influenced by both the length of the first culture phase and the stage at which embryos were explanted. This culture method may be useful for teratological tests, since in the first phase of culture, concentrations of test substances and the time of exposure can be exactly adjusted, and in the second phase, the embryo is allowed to develop quite normally, under conditions similar to those in ovo.

162
Parsons JF, Rockley J, Richold M. IN VITRO MICROMASS TERATOGEN TEST: INTERPRETATION OF RESULTS FROM A BLIND TRIAL OF 25 COMPOUNDS USING THREE SEPARATE CRITERIA. Toxicology In Vitro 1990;4(4-5):609-11.

Results are presented on an inter-laboratory study to validate the Micromass Assay by testing compounds blind using a similar protocol, with an assessment of results for 25 compounds tested without S-9 mix. Four of these were co-tested with S-9 mix. Three separate sets of criteria have been proposed (Flint, 1986 and 1987; Flint and Orton, 1984) for interpreting the results for

teratogenic hazard from in vitro data using IC50 values: (i) the 'less than 500 ug/mL rule', (ii) the 'less than 50 ug/mL rule' and (iii) the 'specific inhibition of cell differentiation 2-fold rule'. The data were decoded and assessed using criteria

established for their sensitivity, specificity and accuracy. In the application of the test, it is suggested that if there was an indication of teratogenic hazard using the '2-fold rule' the compound should be rejected without recourse to animal testing. In its present form the assay cannot be used to unequivocally identify non-teratogens.

163
Hendrickx AG, Binkerd PE. NONHUMAN PRIMATES AND TERATOLOGICAL RESERACH. J Med Primatol 1990;19(2):81-108.

Nonhuman primates were first recognized as models for the study of developmental toxicity (teratology) following the thalidomide tragedy. Since they have played important roles in testing of drugs for human safety and as models for studying specific malformations commonly seen in children. Although in vitro and alternative test systems using lower animal forms or simplified test systems have been incorporated into developmental toxicity studies, whole animal testing will be required for the foreseeable future because of the complex relationship of the maternal/embryo-fetal/placental unit. The nonhuman primate will be particularly valuable in addressing problems of safety when equivocal results are experienced in other commonly used laboratory species.

164
Brown LP, Lewis DF, Flint OP, Orton TC, Gibson GG. TERATOGENICITY OF PHENYLHYDANTOINS IN AN IN VITRO SYSTEM: MOLECULAR ORBITAL-GENERATED QUANTITATIVE STRUCTURE-TOXICITY RELATIONSHIPS. Xenobiotica 1989 Dec;19(12):1471-81.

The ability of 20 mono- and di-phenylhydantoin derivatives to inhibit differentiation of rat embryo mid-brain and limb bud cells in culture has been used as an index of the teratogenic hazard represented by these compounds. Molecular orbital calculations on these compounds, using the MINDO-3 (modified intermediate neglect of differential overlap) and CNDO-2 (complete neglect of differential overlap) methods, were combined with indices of teratogenicity in the two cell types, to generate

a coherent structure-toxicity relationship. Overall, the data emphasize the ability of electronic structural calculations to identify chemical descriptors of toxicity.

165
Uphill PF, Wilkins SR, Allen JA. IN VITRO MICROMASS TERATOGEN TEST: RESULTS FROM A BLIND TRIAL OF 25 COMPOUNDS. Toxicology In Vitro 1990;4(4-5):623-6.

The results obtained by Huntingdon Research Centre participating in a blind trial of the micromass assay for the prediction of teratogenic potential are presented. Twenty-five coded compounds were tested without S-9 mix using a pre-agreed protocol; three compounds were later tested with S-9. The data were assessed for sensitivity, specificity and accuracy using three separate sets of criteria based on either concentration (the less than 500 ug/mL rule (i) and the less than 50 ug/mL rule (ii)) or specific inhibition of cell differentiation at relatively non-cytotoxic concentrations (the 2-fold rule (iii)). The best in vivo/in vitro correlation was obtained using the 2-fold rule; the less than 500 ug/mL rule was the most sensitive but gave a high false positive rate and the less than 50 ug/mL rule was of low overall accuracy (60%).

166
Johnson EM, Newman LM, Fu LJ. AN IN VITRO ASSAY FOR TERATOGENS WITH CULTURES OF RAT EMBRYO MIDBRAIN AND LIMB BUD CELLS. Toxicol Appl Pharmacol 1989 Jun 1;99(1):173-80.

No abstract.

167
Tsuchiya T, Nakamura A, Iio T, Takahashi A. SPECIES DIFFERENCES BETWEEN RATS AND MICE IN THE TERATOGENIC ACTION OF ETHYLENETHIOUREA: IN VIVO/IN VITRO TESTS AND TERATOGENIC ACTIVITY OF SERA USING AN EMBRYONIC CELL DIFFERENTIATION SYSTEM. Toxicol Appl Pharmacol 1991 Jun 1;109(1):1-6.

In vivo/in vitro studies on rats showed that ethylenethiourea inhibited the differentiation of midbrain cells more severely than that of limb bud cells. In in vitro studies using midbrain cell cultures, ethylenethiourea concentrations inhibited the production of differentiated foci by 50% in mouse

cells, a rate 11-fold higher than that in rat cells. Differentiation of rat midbrain cells was also inhibited by the serum samples prepared from rats or mice dosed with up to 200 mg/kg of ethylenethiourea. However, differentiation of mouse cells was not inhibited by these animal serum samples. The concentration of ethylenethiourea in rat sera was only 2-fold higher than that in mice sera at 2 hr after dosing with 200 mg/kg. Therefore, the different sensitivity of the midbrains of these two species may be one reason that ethylenethiourea is teratogenic in rats but not in mice.

168
Ward SJ, Newall DR. THE MICROMASS TEST: IS IT SUBJECT TO STRAIN VARIATION? Toxicology In Vitro 1990;4(4-5):620-2.

Strain difference in the teratogenic response in vivo is an important consideration in the design and interpretation of regulatory studies. The importance of strain difference in vitro has been examined using all trans-retinoic acid. Effects on development of the forelimb in vivo and on differentiation and survival of embryonic limb bud and midbrain cells in vitro were compared for three strains of rat, Allen & Hanbury Albino (AHA), Random Hooded (RH) and Alderley Park (AP). The findings suggested that for retinoic acid, the micromass test is subject to strain variation, although this had no effect on the prediction of teratogenic potential. Furthermore, the order of susceptibility of the three strains examined was the same in vivo and in vitro. Target tissue sensitivity may play an important part in strain variation in the teratogenic response, although differences in vitro were much more marked than those seen in vivo.

169
Ribeiro PL, Faustman EM. EMBRYONIC MICROMASS LIMB BUD AND MIDBRAIN CULTURES: DIFFERENT CELL CYCLE KINETICS DURING DIFFERENTIATION IN VITRO. Toxicology In Vitro 1990;4(4-5):602-8.

Rat embryo micromass limb bud (LB) and midbrain (CNS) cultures have been proposed as a screening system for teratogenic potential as well as a model system for differentiation. To further characterize these in vitro differentiating cultures, their population kinetics have been examined. Cellular proliferation, cell cycle kinetics and cell differentiation were monitored at days 1, 2 and 5

in culture. For cell cycle analysis, cellular DNA was stained with 4,6-diamidino-2-phenyl indole and nuclei were analysed by flow cytometry. Cell viability was assessed using trypan blue and differentiation was monitored using haematoxylin (CNS) or alcian blue (LB) stain. Characterization of the cellular kinetics of the two widely used embryo cell culture systems studied, should help to delineate potential sites and mechanisms of developmental toxicity.

170
Regan CM, Gorman AM, Larsson OM, Maguire C, Martin ML, Schousboe A, Williams DC. IN VITRO SCREENING FOR ANTICONVULSANT-INDUCED TERATOGENESIS IN NEURAL PRIMARY CULTURES AND CELL LINES. Int J Dev Neurosci 1990; 8(2):143-50.

To establish inherent potential for the induction of neural tube defects the ability of selected anticonvulsant agents to interfere with cell division has been established in vitro using an antiproliferative assay in clonal cell lines and a cytotoxicity assay using primary cultures of cerebral cortex neurons at different stages of development. In order to evaluate the relative toxicities of these agents their in vitro effects were determined at 2-3 times the plasma therapeutic level. When compared to epidemiological and animal study data, agents which inhibited cell proliferation within twice therapeutic concentration were consistently associated with major neural tube malformations. Thus assessment of antiproliferative activity of anticonvulsant drugs may be one criterion for identification of teratogenic potential during neurulation.

171
Steele CE. WHOLE EMBRYO CULTURE AND TERATOGENESIS. Hum Reprod 1991 Jan;61:144-7.

Whole embryo culture (WEC) is under evaluation in numerous laboratories to determine the role it should play in teratogen testing. There is general agreement that its role is to complement the Segment II Teratology studies required by regulatory authorities. Currently it is used as a 'screen' in order to minimize the number of compounds used in animal tests and also to study mechanism(s) of teratogenesis. This brief review focuses on the study of retinoid teratogenicity in WEC in order to illustrate these points.

172
Nakaura S, Tanaka S, Kawashima K, Takanaka A, Djajalakasana S, Huang ML. IN VITRO TERATOGENICITY TESTING USING THE RAT EMBRYO CULTURE SYSTEM: (2) EFFECTS OF ETHYLENETHIOUREA ON RAT EMBRYONIC DEVELOPMENT IN VITRO AND IN VIVO.

Teratology 1989;40(6):684.

Teratogenic effect of ethylenethiourea (ETU) was examined in rat embryos developing in vitro and in vivo. In both the in vitro and in vivo experiments ETU caused a retardation of the embryonic development as measured by yolk sac diameter, crown-rump length, number of somites and protein content. A variety of malformations such as stunted brain, open neural tube and small and irregular somites were produced. The incidence of malformations increased with an increased concentration or dose of ETU. The malformations produced in the in vitro experiment were comparable to those in in vivo. These results suggest that the in vitro rat embryo culture method is useful for in vitro teratogenicity testing, and further, this system has the potential to provide useful information about teratogenic mechanisms.

173
Tsuchiya T, Matuoka A, Sekita S, Hisano T, Takahashi A, Ishidate M Jr. HUMAN EMBRYONIC CELL GROWTH ASSAY FOR TERATOGENS WITH OR WITHOUT METABOLIC ACTIVATION SYSTEM USING MICROPLATE. Teratogenesis Carcinog Mutagen 1988;8(5):265-72.

In vitro microassay as a means to screen for teratogens was used on the cancer chemotherapeutic agents sterigmatocystins and benzimidazoles using human embryonic palatal mesenchymal (HEPM) cells.

174
Bechter R, Terlouw G DC, Tsuchiya M, Tsuchiya T, Kistler A. TERATOGENICITY OF CAROTINOIDS (RETINOIDS) IN THE RAT WHOLE EMBRYO CULTURE. Arch Toxicol 1992; 66(3):193-197.

Structural modifications of the carotinoid molecule RO 13-7410 led to changes in their teratogenic potency of more than five orders of magnitude in mice in vivo and in micromass cultures of rat embryonic limb bud cells (Kistler et al. 1990). Five of the retinoids were selected and tested in rat whole embryo culture to

determine the suitability of this in vitro test system in identifying potentially non-teratogenic derivatives among this class of chemicals. It was concluded that whole embryo culture system is a useful tool for the preliminary testing of retinoids for teratogenicity.

175
Bournias-Vardiabasis N. DROSOPHILA MELANGOASTER EMBRYO CULTURES: AN IN VITRO TERATOGEN ASSAY. Alternatives Lab Anim 1990;18:291-300.

An in vitro teratogen assay has been developed that uses Drosophila embryo cell cultures. Endpoints selected in this system to assess the teratogenic potential of any agent (physical or chemical) involves detection of interference with normal muscle and/or neuron differentiation, induction of heat shock (stress) proteins, and inhibition of normal neurotransmitter levels. Current studies involve use of reporter gene technology (protein fusions) to identify teratogenicity. Results so far suggest that the Drosophila assay is capable of accurately establishing if a particular agent tested can act as a teratogen by a variety of appropriate endpoints (morphological, biochemical, molecular). This assay can be used as a teratogen screen, and in mechanistic studies of abnormal development, gene involvement in teratogenic resistance, and the possible role of heat shock proteins in preventing birth defects.

176
Nito S, Ariyuki F, Nakayama Y. A NEW IN VITRO SCREENING METHOD FOR TERATOGENS USING HUMAN EMBRYONIC PALATAL MESENCHYMAL CELLS. Congenital Anom 1991; 31(4):329-336.

To establish an in vitro screening assay system for cleft palate-inducing teratogens, tested were 31 teratogenic and 10 nonteratogenic compounds using cultured human embryonic cells. We examined whether cleft plate-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 proliferatio of HEPM cells. These experimental results indicate that teratogens which induce cleft palate in vivo preferentially inhibit the proliferation of embryonic palatal mesenchymal cells. Data

also indicate that in vitro screening using HEPM and MRC-5 cells is useful for detecting the cleft palate-inducing ability of chemicals.

177
Reinhardt CA, Bienz A, Romano-Diethelm T, Wyle-Gyurech GG. IN VITRO DIFFERENTIATION OF EMBRYONIC CHICK BRAIN CELLS: DEVELOPMENT OF A NEUROTERATOLOGY TEST SYSTEM. Experientia 1991 Feb;47:A5.

To develop a model for potential neurotoxicity and teratogenicity, chick brain cells (embryonic day 7, ED7) were mechanically dissociated and then cultured for upwards of several months. Differentiation of nerve and glial cells (in petridishes, monolayers; and suspension cultures as reaggregates) were monitored with monoclonal antibodies against 68kD neurofilament protein (anti-NF) glial fibrillary acidic protein (anti-GFAP) and tyrosine hydroxylase as well as by monitoring the protein synthesis pattern by 2D-gel electrophoresis. Anti-NF stains neurons in vitro as they differentiate morphologically. In reaggregates a stable differentiation of nerve cells could be observed by intensive anti-NF staining for as long as 3 wk to 5 wk in culture. The expression of NF in nerve cells is proposed to be used as a sensitive cytotoxicity end-point whereas GFAP expression can

serve as an endpoint for monitoring differentiation of neural tissue (Wyle & Reinhardt, Toxicol. In Vitro, in press). Monitoring functional parameters of the dopaminergic system (such as activity of tyrosine hydroxylase) and other general endpoints (protein metabolism, cytotoxicity) will be combined to evaluate the in vitro toxic potential of known neuroteratogens such as psychoactive drugs and analgesia.

178
Estus S, Blumer JL. ROLE OF MICROTUBULE ASSEMBLY IN PHENYTOIN TERATOGENIC ACTION IN THE SEA URCHIN (ARBACIA PUNCTULATA) EMBRYO. Mol Pharmacol 1989 Nov;36(5):708-15.

The role of microtubule assembly in phenytoin induced (5-5-diphenyl-hydantoin) teratogenic activity in the sea urchin embryo was studied. Zygotes were exposed to phenytoin or one of several phenytoin analogs within 15 min of fertilization and the frequency of the resultant malformations was assessed at the cleavage and late gastrula (prism) stages. Concomitant studies of drug uptake into zygotes and drug effects on both

microtubule assembly in vitro and spindle morphology in situ were also performed. Phenytoin, 5-p-methylphenyl-5-phenylhydantoin, and 5-p-methoxyphenyl-5- phenylhydantoin were teratogenic (approaching 100% affected embryos) at both developmental stages were concentrated rapidly by the zygotes, and induced a shortened mitotic spindle in situ. In a separate in vitro system using porcine brain microtubular protein, these analogs were shown to inhibit microtubule assembly directly. Another analog, 5-p-hydroxyphenyl- 5-p'-methylphenylhydantoin was not teratogenic at concentrations up to the limit of its solubility. If this analog were as potent inside the cell as either phenyltoin or 5-p-hydroxyphenyl-5-phenylhydantoin, the intracellular concentrations achieved should have been sufficient to induce abnormal cleavage. Thus, the lack of teratogenic efficacy of this analog was correlated with its observed lack of effects on either microtubule assembly in vitro or spindle formation in situ. Overall, these studies are consistent with a hypothesis that phenytoin may induce abnormal development in the porcine brain microtubule protein system by a direct inhibition of microtubule assembly.

179
Abbott BD, Buckalew AR. EMBRYONIC PALATAL RESPONSES TO TERATOGENS IN SERUM-FREE ORGAN CULTURE. Teratology 1992;45(4):369-382.

This study examined development of rat, mouse, and human embryonic palates in submerged, serum-free organ culture. The concentration- response profiles for retinoic acid (RA), triamcinolone (TRI), hydrocortisone (HC), dexamethasone (DEX), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were examined and the mechanisms of clefting in vitro were compared to observed in vivo responses. The present study also demonstrates that serum-free organ culture supports development of mouse, rat, and human palatal explants. The study demonstrated the capacity of this organ culture system to model palatogenesis for several species, and to distinguish between various mechanisms of clefting as presented through selected model compounds. This biological model should be useful for exploring mechanisms of activity at a cellular and molecular level.

180
Fitzgerald MP, Daston GP, Elmore E. OPTIMIZATION OF

THE CHICK EMBRYO RETINA CELL ASSAY TO SCREEN TERATOGENS. Teratology 1990 May;41(5):557.

In response to a growing need for reliable short term in vitro assays to test the potential teratogenicity of compounds, the Chick Embryo Retina Cell Assay was investigated. This in vitro screening system assesses the effect of potential teratogens on three endpoints: inhibition of cell aggregation, growth inhibition and inhibition of cortisol induced differentiation. Test chemicals may interfere with any one of these endpoints and the endpoint mechanisms are not necessarily related. This method also allows for the assessment of all three endpoints from the same treated cell population. All tests are performed using neural retinal cells from 7.0 +/- 0.5 day White Leghorn chick embryos. The effects of serum lot, cell density, aggregate size, pH and shaker speed on assay variability were evaluated. Differences in the optimal shaker speeds for aggregation and differentiation were observed. All of the chemicals affected at least one endpoint at concentrations less than 1mM. An evaluation of the optimized Chick Embryo Retina Cell Assay to determine its accuracy in predicting the teratogenicity of coded agents is on-going.

181
George-Weinstein M. IN VITRO ASSAYS OF CELL ADHESION: APPLICATION TO TERATOGENICITY TESTING. In Vitro Methods in Developmental Toxicology 1990;(0):113-28.

No abstract.

182
Hales BF. TERATOGENICITY. Frazier, J. M. (ED.). IN VITRO TOXICITY TESTING: APPLICATIONS TO SAFETY EVALUATION. VIII+299P. Marcel Dekker, Inc.: New York, New York, USA; Basel, Switzerland. Illus. ISBN 0-8247-8614-9.1992;0(0):205-220.

No abstract.

TOXICOLOGY (GENERAL)
183
Zbinden G. REDUCTION AND REPLACEMENT OF LABORATORY ANIMALS IN TOXICOLOGICAL TESTING AND RESEARCH. Interim report 1984-1987. Biomed Environ Sci 1988 Jun;1(1):90-100.

The reasons to support reduction and replacement of laboratory animals are advancing rapidly in basic biomedical research, and why in industrial toxicology progress is much slower, are analyzed. Encouraging developments concerning acceptance of new concepts in acute toxicity testing by various regulatory agencies are reviewed. The possibilities of reducing animal use in toxicology by application of toxicological screening procedures are described. Screening tests under development include an operant conditioning technique to detect adverse drug interactions with ethanol and a procedure for the detection of nephrotoxic properties. The successful completion of a collaborative program designed to upgrade toxicity testing with contraceptive steroids and to abolish the 7-year beagle and 10-year monkey studies is reported. New tests developed at the Institute are described. Cell culture methods under development include a culture system of chick brain, retina, and meniges cells for the study of neurotoxic chemicals and neurobehavioral teratogens, primary hepatocyte cultures for the study of drug effects on DNA and protein synthesis and ploidy, using flow cytometry, and various in vitro models for the assessment of genotoxic and tumor-promoting activities and malignant cell transformation. The problem of analgesic treatment of animals with chronic pain was investigated.

184
Tanimura T. PERSPECTIVES ON TESTING CHEMICALS FOR REPRODUCTIVE AND DEVELOPMENTAL TOXICITY. Teratology 1990 Sep;42(3):326.

There are four checkpoints to the reproductive and developmental toxicity of chemicals: chemical structure and properties, in vitro experiments including submammalian species, in vivo mammalian tests and human surveys. For pharmaceutical drugs, the three segment studies originating from USFDA 1966 guidelines are requested by all the countries. However, the fundamental policy of the Japanese and Nordic guidelines is different from that of USA and EC. To attain international conformity on the guidelines, scientific discussion with international organizations is necessary, with most likely not a quick resolution of differences. For environmental chemicals, the basic design is almost the same throughout the world consisting of the multigenerational teratogenicity studies. Practical in vitro models are expected to be available for the initial screening of a large number

of environmental chemicals. More efforts should be directed internationally to establish and validate risk assessment procedures. Examination of the scientific validity of tests, studies on mechanisms and pharmacokinetics, and development of qualitative evaluation are important for the better extrapolation of the animal data to humans.

185
Gad SC. RECENT DEVELOPMENTS IN REPLACING, REDUCING, AND REFINING ANIMAL USE IN TOXICOLOGIC RESEARCH AND TESTING. Fundam Appl Toxicol 1990 Jul;15(1):8-16.

Significant progress has been made in replacing animals in toxicology/safety assessment with in vitro systems, in reducing the number of animals used, and in refining how they are used. Review of annual reports of the numbers of animals used in testing in the United States, the United Kingdom, and Japan shows a continuing reduction in the numbers for all species. Multiple in vitro systems have been developed for screening/testing for eye and skin irritation, skin sensitization, teratology, and other endpoints and a scientific consensus has been formed on requirements and process for validation. However, the use of these test systems in place of existing in vivo tests is minimal. At the same time, innovative designs have been developed (and are in wide use) for in vivo tests which reduce both the numbers and the pain and distress of animals used in testing. Progress and dialogue continue on modification of both U.S. and international requirements and guidelines for testing, and for defining an "approval" process for alternatives and innovations.

VALIDATION TESTS
186
Renault JY, Melcion C, Cordier A. LIMB BUD CELL CULTURE FOR IN VITRO TERATOGEN SCREENING: VALIDATION OF AN IMPROVED ASSESSMENT METHOD USING 51 COMPOUNDS. Teratogenesis Carcinog Mutagen 1989;9(2):83-96.

Rat embryo limb bud cells multiply and undergo chondrogenesis in micromass culture. Teratogenic agents may be identified from their inhibition of chondrogenesis, which is quantified by determination of cartilaginous foci number or proteoglycan production. In other in vitro systems, the detection is based on their ability to affect cell proliferation. So far, these methods have failed to distinguish among true inhibition of differentiation, inhibition of cell proliferation, and nonspecific

cytotoxicity. The improved technique involves simultaneous measurement of cartilage synthesis and cell multiplication. Improvements have increased the specificity of the micromass culture test. Validation was performed using 51 compounds. Compounds were classified according to their inhibitory activity and their active concentration. The sensitivity of the test was 61%; the specificity, 100%; and the final accuracy, 75%. The method is fully miniaturised, automated, and computerised, allowing numerous compounds to be rapidly tested at very low cost.

187
Weber E. PHARMAKOLOGISCHE UNTERSUCHUNGEN IN VITRO - ALTERNATIVEN ZUM TIERVERSUCH. ABSCHLUSSBERICHT. (PHARMACOLOGICAL INVESTIGATIONS IN VITRO - ALTERNATIVES TO ANIMAL EXPERIMENTS. FINAL REPORT). Bundesministerium fuer Forschung und Technologie, Bonn (Germany, F.R.). Govt Reports Announcements & Index (GRA&I), Issue 03, 1992.

The aim of the project 'Pharmacological Investigations In Vitro - Alternatives to Animal Experiments', was to develop, 'pain-free' cell and tissue tests which could be utilized for the screening of potential drugs. The in-vitro models upon which the research effort was concentrated were intended to identify anti-inflammatory and immunoregulatory compounds, antiatherosclerotic compounds, compounds active as cardiovascular and psychotropic agents, antiparasitic compounds. For each of these indications a variety of in-vitro tests were investigated. It proved possible to demonstrate a good correlation between results of in vitro and in vivo investigations for some of the cell culture models utilized. The mechanistical predictive potential, the small amount of test compound required, the large screening capacity and the rapid and inexpensive nature of these tests offer advantages over animal experiments.

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Ghaida J, Merker HJ. EFFECTS OF CYCLOPHOSPHAMIDE AND ACROLEIN IN ORGANOID CULTURES OF MOUSE LIMB BUD CELLS GROWN IN THE PRESENCE OF ADULT RAT HEPATOCYTES. Toxicol In Vitro 1992;6(1):27-40.

Effects were evaluated of cyclophosphamide (CPA) and its metabolite, acrolein, on chondrogenesis in organoid cultures of mouse limb bud mesenchymal cells co-cultured with non-enzymatically isolated adult rat hepatocytes. The studies were conducted with or without the simultaneous addition of 2-mercaptoethanesulphonic acid sodium (mesna) or glutathione (GSH). Alcian blue

Oct;13(Oct 18):210-214. (7 REFS)

The release and penetration of cream and ointment formulations of 0.5% anthralin were studied in vitro; one cream formulation was compared with a commercial product (Psoricreme) in rabbits and in 27 patients with chronic plaque psoriasis using a double blind

randomized design. The in vitro method was not suitable for predicting in vivo release and penetration. In rabbits the degree of skin irritation produced was comparable for both preparations. In patients the 2 creams were equally effective for the treatment of psoriasis and showed the same incidence of side effects. It was concluded that the efficacy and toxicity of 0.5% anthralin cream are comparable to those of a commercial formulation in patients with psoriasis; in vitro results were not useful for predicting in vivo effects.

195
Pagano G, Trieff NM. USE OF ECHINOIDS IN IN VITRO EMBRYOTOXICITY AND TERATOGENICITY STUDIES. COMMENTS. Toxicol in Vitro 1992;6(2):177-9.

The review on in vitro embryotoxicity and teratogenicity studies by P. W. J. Peters and A. H. Pieroma (ibid 1990, 4, 570-576) provided a comprehensive list of in vitro systems for testing developmental toxicity, and highlighted some major issues surrounding the concept and utilization of systems. The authors made a questionable statement by claiming that sea urchins are tested for cell replication and contacts in cleavage, which is

incomplete. The author's statement that nonmammalian systems have potential merit but require careful review and detailed validation may be accepted as a challenge to advance the present state of the art. It would be desirable for authors of future review papers dealing with topics as crucial as test-systems validation to ensure that they are supported by recent literature, not confined to the authors' own field, with the aid of widely available information retrieval systems.

196
Purchase IF. PRINCIPLES FOR THE VALIDATION AND USE OF IN VITRO TOXICOLOGICAL TECHNIQUES. Teratology 1990 Sep;42(3):326.

Enthusiastic scientists can and have suggested numerous

in vitro methods as replacements for established regulatory studies. Tests for mutagenicity and teratogenicity are excellent examples of this phenomenon. In spite of the legitimate criticisms of in vivo toxicological methods, any replacement must be shown to be at least as effective and robust before it can be introduced into general use. Formal intra- and inter-laboratory validation studies should be used for this purpose. Even when successful validation has been achieved there are important issues to be resolved before widespread acceptance. No in vitro screening test is 100% accurate and this presents problems in their deployment and use. In particular, the characteristics of batteries of tests should be considered before applying them to screening large numbers of chemicals.

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Anderson D. IN VITRO MODELS. Drug Safety 1990;5 Suppl 1:27-39.

The development of in vitro models is advancing rapidly, with the application of cell culture methods as an alternative to animals in toxicological screening; non-cellular systems are also being used. Any screening test, whether for detecting irritants, carcinogens or teratogens should be well validated against known animal studies. Some validated test systems have been used for several years to detect genotoxins and are acceptable to many regulatory authorities. This is not the case for other in vitro systems, although some for skin irritation and corrosion are acceptable to some authorities. A non-comprehensive range of tests to measure responses in ocular, immune, cardiac, vascular, neurological, sensory, hepatic, testicular and embryological systems are described. Some tests are empirical and others much more closely mimic the animal model they represent. Some of the methods described are much closer to appropriate degrees of validation than others.

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Piersma AH, Attenon J, Govers MJ, van Maele-Fabry G, Peters PW, Picard J, Schmid BP, Stadler J, Verhoef A, Verseil C. RODENT POSTIMPLANTATION EMBRYO CULTURE: INTERLABORATORY VALIDATION AS A SCREENING TEST FOR