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

A Bibliography with Abstracts

To Assist In:

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

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

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

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Suggestions and comments are welcome.

BRAIN

1

Tsuchiya T. METHOD OF MICROMASS CULTURE OF MIDBRAIN CELLS AND ITS APPLICATION TO IN-VITRO MECHANISTIC STUDY. 31ST Annual Meeting Of The Japanese Teratology Society, Izumo, Japan, July 11-12, 1991. Congenital Anom 1991;31(3):222.

No abstract.

2

Davies DL, Ross TM. LONG-TERM ETHANOL-EXPOSURE MARKEDLY CHANGES THE CELLULAR COMPOSITION OF CEREBRAL GLIAL CULTURES. Dev Brain Res 1991;62(2):151-158.

The vulnerability of morphologically distinct glial subpopulations to ethanol toxicity was surveyed in tissue culture. Secondary cultures of rat glia were examined at intervals during 56 days of ethanol treatment for changes in growth and cellular characteristics. Relative to control cultures, the ethanol-treated cultures established a consistent and dose-dependent suppression in cell number. In control cultures, a subpopulation of process-bearing cells was

acquired gradually during the first 3 weeks in culture. The majority of these process-bearing cells were considered to be oligodendrocytes due to their position above the astrocytic carpet and by the immunocytochemical localization of galactocerebroside. Exposure to 0.5% ethanol markedly suppressed the acquisition of process-bearing cells. Results suggest a possible differential sensitivity of oligodendrocytes and of their precursors to ethanol toxicity at elevated (0.5% w/v) concentrations.

CARCINOGENESIS

3

Lijinsky W. NON-GENOTOXIC ENVIRONMENTAL CARCINOGENS. Journal of Environmental Science and Health. Part C: Environmental Carcinogenesis Reviews C8(1):45...1991.

Nongenotoxic environmental carcinogens were discussed. The basic hypothesis that cancer is caused by somatic mutations has led to the assumption that carcinogens must be mutagenic and that they induce cancer as a result of mutational changes induced in DNA. A considerable number of carcinogens exist that are not mutagenic. It was noted that satisfactory explanations for the carcinogenic activity of nonmutagenic substances have not been offered. The lack of

genotoxicity of some carcinogens may be related to biological differences between cells in culture and whole organisms. Carcinogenesis as observed in laboratory animal studies and epidemiological surveys was considered. Mutagenesis was discussed. The Ames/Salmonella assay was described. The characteristics of nongenotoxic carcinogens were reviewed. Important nongenotoxic environmental carcinogens were summarized. Possible mechanisms for nongenotoxic carcinogenesis were discussed. Genotoxicity should be regarded as an additional, but not essential property of some carcinogens. Assessing the human cancer risk of nongenotoxic carcinogens was discussed. It was noted that bacterial mutagenesis assays and other short term bioassays cannot be used for assessing the risk of nongenotoxic carcinogens. Risk assessments should be based on evaluating the risk of carcinogens as a whole, not distinguishing between nongenotoxic and genotoxic carcinogens, starting with the results of laboratory animal tests.

4

Gebhardt R. HISTOCHEMICAL APPROACHES TO THE SCREENING OF CARCINOGENS IN-VITRO in Graumann, W. (ED.). Progress in Histochemistry and Cytochemistry, Vol. 23, No. 1-4, 1991.

No abstract.

5

Marczynska B, Khoobyarian N, Chao TS, Tao M. PHORBOL ESTER PROMOTES GROWTH AND TRANSFORMATION OF CARCINOGEN-EXPOSED NONHUMAN PRIMATE CELLS IN VITRO. Anticancer Res 1991;11(5):1711-7.

Kidney cells established in vitro from a white-lipped marmoset (106) were exposed to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) alone or in combination with 12-O-tetradecanoyl-phorbol-13-acetate (TPA). Low (0.1 micrograms/ml, 4 times), intermediate (1 microgram/ml) and high (1 microgram/ml, 4 times) doses of MNNG resulted in 100%, 50% and 2.8% of cell survival, respectively. High and low doses of MNNG had no effect on cell transformation. Upon exposure of cells to an intermediate dose of MNNG, 106 cells evolved into permanent cell line, 106-1M. However, the cells retained normal morphology and anchorage dependence. Chronic applications of TPA (0.1 micrograms/ml, 13 times) promoted 106-1M cells to morphological transformation and anchorage-independent growth but not to tumorigenicity in nude mice (106-1MT

cell line). Chromosome analysis revealed only numerical changes in 106 cells and both numerical and structural aberrations in transformed 106-1MT cells. These changes in marmoset cells usually reflected cell culture instability leading to either senescence or to longer survival of cells in vitro.

6

Kerr DJ, Smart HI. THE RELATIONSHIP BETWEEN CYTOTOXIC DRUG EXPOSURE AND TUMOR CELL KILL, IN VITRO AND IN VIVO. *In Vivo* 1991;5(4):385-8.

Doxorubicin has been shown to be more effective against MGH-U1 bladder carcinoma cells grown in monolayer than spheroid. In vitro clonogenic cell survival curves have been replotted against the area under the concentration-time curve (AUC) for drug exposure and fitted to a Hill plot to derive the parameters E max (max. possible cell kill) and C50 (drug exposure resulting in half the maximum cell kill). The plasma AUC following i.p. administration of doxorubicin to nude mice was measured using a sensitive and specific HPLC assay and combined with the in vitro cell survival parameters to predict the clonogenic cell survival in MGH-U1 xenografts. The Hill parameters from the spheroid model are better predictors of xenograft clonogenic cell survival than the monolayer parameters. It is possible to predict clonogenic cell survival in solid tumors on the basis of the pharmacokinetics of cytotoxic drug exposure using a mathematical model based on clonogenic cell kill in vitro.

7

Stoica G, Jacobs R, Koestner A, O'Leary M, Welsch C. ENU-INDUCED IN VITRO NEOPLASTIC TRANSFORMATION OF RAT MAMMARY EPITHELIAL CELLS. *Anticancer Res* 1991; 11(5):1783-1792.

Normal mammary epithelial cells, originating from female Sprague-Dawley rats, were grown in Dulbecco's Modified Eagles Medium containing 25% horse serum and hormone supplements. Once established as an epithelial cell culture, the cells were treated with N-ethyl-N-nitrosourea (ENU) in various doses (25-500 µg/ml) to study the process of in vitro mammary epithelial cell neoplastic transformation. The rat mammary epithelial cells, after a period of approximately 30 days post-ENU exposure, showed marked proliferation of morphologically altered cells which formed multi-layered colonies. Subsequently, these cells acquired the capacity to form colonies in soft

agar and eventually produced a high yield of palpable tumors when inoculated into newborn female isologous hosts or female athymic nude mice. The immediate effect of ENU on these cells was monitored by measurement of cellular DNA content, unscheduled DNA synthesis, cell proliferation and chromosomal aberrations. Single chromatid breaks, isochromatid breaks, chromosomal exchanges, multiple chromosomal breaks and double minutes were among the chromosomal aberrations seen in EMU-treated cells. The ENU-induced model of in vitro neoplastic transformation of rat mammary epithelium as described in this communication appears to provide a good model of the systematic study of the early critical cellular events prerequisite to this carcinogenic process.

8

Ino K, Goto S, Kosaki A, Nomura S, Asada E, Misawa T, Furuhashi Y, Mizutani S, Tomoda Y. GROWTH INHIBITORY EFFECT OF BESTATIN ON CHORIOCARCINOMA CELL LINES IN VITRO. *Biotherapy* 1991;3(4):351-7.

Bestatin, one of the biological response modifiers (BRMs), is an inhibitor of aminopeptidase B (AP-B), leucine aminopeptidase (LAP) and aminopeptidase M (AP-M). Investigated was the direct effect of bestatin on the growth of cancer cells in vitro using established four choriocarcinoma cell lines. In vitro chemo-sensitivity was evaluated by the succinate dehydrogenase inhibition (SDI) test. Bestatin showed the growth-inhibitory effect on all the choriocarcinoma cell lines dose-dependently, especially on NaUCC-4 cells. Cytotoxicity of actinomycin D on the choriocarcinoma cells was significantly enhanced by combination with bestatin. Results of the study suggest that bestatin has not only an indirect host-mediated anti-tumor activity, but also a direct growth-inhibitory effect on some kinds of cancer cell lines.

9

Benigni R. THE ABILITY OF SHORT-TERM TESTS TO PREDICT CARCINOGENICITY CAN BE SUMMARIZED IN A SINGLE INDEX. *Journal of Toxicology and Environmental Health* 1991; 34(1):27-37. (16 REFS)

A new procedure using Relative Operating Characteristics (ROC) analysis was described for quantitating performance of short term tests (STTs) in discriminating between carcinogens and noncarcinogens. The analysis was applied to a comparison of rodent carcinogenicity data for 64 compounds with their

in-vitro genotoxicity in four STTs. STTs included Salmonella-typhimurium mutagenicity, mutagenesis in mouse lymphoma cell line L5178Y, chromosomal aberration in Chinese-hamster ovary (CHO) cells and sister chromatid exchanges in CHO cells. The discriminatory capabilities of the four STTs were assessed by linear discriminant analysis. Outcomes were represented as two by two contingency tables, which were then plotted as points on a graph using false positive proportion as the x-axis and true positive proportion as the y-axis. Analysis of the resulting curve produced a value reflecting accuracy. The accuracy of the battery was determined to be 0.69. This was consistent with previous analyses that indicated an accuracy of about 60% for each STT to predict rodent carcinogenicity of these compounds. It was concluded that test results demonstrate the possibility of summarizing carcinogenicity prediction performance of STTs in a single index.

CELL CULTURE

10

Chang MJW. UV ABSORPTION AS AN APPROXIMATION FOR CELL NUMBER IN IN VITRO TOXICITY TESTING. Bulletin of Environmental Contamination and Toxicology 1991;47(2):190-197. (13 REFS)

The use of ultraviolet absorption as an approximation for cell number in in-vitro toxicity testing was described. Three different cell cultures (Chinese hamster ovary cells, human lung fibroblasts, and normal human epidermal keratinocytes) were characterized biochemically. The ultraviolet absorption at 260 nanometers (A260) of a cell solution was observed to be consistently proportional to the total number of cells involved. Cell growth curves monitored by A260 closely resembled those monitored by cell number or protein content. It was concluded that A260 is a good approximation to represent number of cells for in-vitro study. Some advantages of A260 are that there is no requirement for a special reagent, a relatively small number of cells is needed per sample, and the procedure is so simple that only one step is involved in obtaining an A260 reading.

11

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

A quantitative in vitro transformation assay has been

developed for the first time using primary rat kidney epithelial (RKE) cells. Morphologically transformed colonies gave rise to cell lines with indefinite growth capacity and neoplastic potential. The assay represents the first in vitro model for studying mechanisms of chemical transformation of normal kidney epithelial cells and may also be useful as a screen for identifying potential renal carcinogens.

12

Guillot R. [CELL CULTURES. COMPLEMENTARY OR ALTERNATIVE METHODS TO ANIMAL EXPERIMENTATION]. J Pharm Belg 1991,May-Jun;46(3):197-200.

In vitro methods, which appeared at the beginning of the century, have had exponential development for ethical and technological reasons. They can be used for studies concerning intracellular, intercellular or environmental activities. Despite limiting factors, these techniques offer certain advantages which have to be optimized in order to be validated. They are actually complementary and used for exploratory purposes. In the future, they could replace partially, but not completely, some in vivo experiments.

13

Tarkington M, Sommers CL, Gelmann EP, Tefft MC, Lynch JH. THE EFFECT OF pH ON THE IN VITRO COLONY FORMING ABILITY OF TRANSITIONAL CELL CARCINOMA CELLS TREATED WITH VARIOUS CHEMOTHERAPEUTIC AGENTS: IMPLICATIONS FOR IN VIVO THERAPY. J Urol 1992;147(2):511-3.

Extracellular pH may affect the sensitivity of cancer cells to chemotherapy agents. In an attempt to maximize the conditions for chemotherapy treatment of transitional cell carcinoma tested was the effect of pH on sensitivity of MGH-U3 transitional cell carcinoma cell line to thiotepa, doxorubicin, and mitomycin c in vitro. The toxicity of each agent tested varied with pH. There was no variation in cell growth in response to pH alone. Significant differences were also observed after treatment with doxorubicin and mitomycin c with a diluent pH of 7.0. This in vitro assay may be useful for clinical application of pH modulation during intravesical chemotherapy.

14

Zelesco PA, Barbieri I, Graves JAM. USE OF A CELL HYBRID TEST SYSTEM TO DEMONSTRATE THAT BENOMYL INDUCES

ANEUPLOIDY AND POLYPLOIDY. *Mutat Res* 1990;242(4):329-335.

In order to confirm the usefulness and generality of the cell hybrid assay system for detection of aneuploidy producing agents, the effect of benomyl on the monochromosomal Chinese-hamster/human hybrid EUBI was tested. A 24 hour treatment of cell cultures with varying doses of benomyl (2, 4, or 8 micrograms/milliliter) or dimethyl-sulfoxide and followed by two rinses with serum free medium and replacement of medium with fresh medium. After a 24 hour recovery period, a cytogenetic analysis of each treated culture was performed. The findings demonstrated a dose dependent increase for the induction of aneuploidy by benomyl and a very greatly increased percentage of polyploid cells at the highest benomyl concentration tested. It was concluded this assay is useful for determining genetic risk associated with human exposure to environmental chemicals.

15

Bean CL, Armstrong MJ, Galloway SM. EFFECT OF SAMPLING TIME ON CHROMOSOME ABERRATION YIELD FOR 7 CHEMICALS IN CHINESE HAMSTER OVARY CELLS. *Mutat Res* 1992; 265(1):31-44.

Choice of harvest time is one of the most important variables in the assessment of whether a compound is clastogenic and in establishing a dose relationship. We examined the effects of sampling time on aberration yield for 7 diverse chemicals in CHO-WBL cells by harvesting at intervals from 9 to 30 h after treatment for 3 h with or without S9 metabolic activation. Data suggest that for most compounds a single harvest time approximately 17-21 h after the beginning of a 3-h treatment is optimal for aberration detection in CHO cells. For most chemicals the maximal aberration yield occurred at a different time for each concentration tested. However, the use of 3 or more closely spaced concentrations, carefully selected to yield up to 50% toxicity, allowed detection of a positive response at a single harvest time for all chemicals tested.

16

Roussakis C, Gratas C, Audouin AF, Le Boterff J, Dabouis C, Andre MJ, Moyon E, Vo NH, Pradal G, Verbist JF. STUDY OF IN VITRO DRUG SENSITIVITY ON A NEWLY ESTABLISHED CELL LINE FROM A PRIMARY BRONCHIAL EPIDERMOID CARCINOMA OF HUMAN ORIGIN (NSCLCN6). *Anticancer Res* 1991;11(6):2239-44.

Studied was a cell line established from a primary non-small-cell lung cancer (non-SCLC) of human origin and characterized by mildly differentiated epidermoid carcinoma, a human karyotype and keratin expression. In vitro, this cell line was mainly sensitive to dactinomycin and mitotic poisons such as Vinca alkaloids. Most chemotherapeutic drugs proved ineffective. Study findings are comparable to previous results in patients who showed 30% objective response and less than 5% complete response regardless of the therapeutic associations used against non-SCLC. The cell line would seem to provide a good model for studying new potentially antitumor substances.

17

Messier AA, Fisher HW. SENSITIVITY OF CULTURED MAMMALIAN CELLS TO OXIDATIVE STRESS: ADAPTATION TO REPEATED EXPOSURES OF HYPERBARIC OXYGEN. Undersea Biomedical Research 1990;17(4):305-322. (36 REFS)

A more rapid method was developed for producing oxygen resistant mammalian cell lines by the repetitive exposure of surviving cells to hyperbaric oxygen (HBO). The cell lines utilized included Chinese-hamster ovary (CHO), mouse neuroblastoma N2A, Chinese-hamster lung V79, and rabbit kidney RK13. The experimental HBO exposure protocol included repeated exposures to 100% oxygen at 6 and 10 atmospheres absolute (ATA) for time periods up to 3 hours, with intervening 1 week recovery periods at normal atmospheric pressure and regrowth of surviving cells to monolayer confluency before reexposure: typically, the entire protocol, including regrowth of the resistant population, required only 3 to 4 weeks. Although all cell lines could be made oxygen resistant, there were qualitative

differences among the cell lines. It was concluded that the ability of the new method to generate oxygen resistant cells in the relatively short time of 3 to 4 weeks provides a considerable time savings over the 12 to 20 months required by previously published studies. The new method not only allows rapid screening of various cell types, but may also result in the discovery of oxygen resistant cell types which may provide insight into the complexity of the adaptation of cells to oxidative stress.

18

Seed TM, Kretz ND, Schlenker RA. RADON EXPOSURE SYSTEM FOR MAMMALIAN CELLS IN CULTURE: DESIGN, OPERATION, AND DOSIMETRY. Health Physics 1991;61(3):349-357. (23 REFS)

A radon exposure system was designed for mammalian cells in culture. The study was initiated on the premise that the toxicity of radon gas could be assessed directly by using the dose dependent, end point specific responses of select types of bronchopulmonary cells, isolated, propagated, and exposed in-vitro. The exposure system was tested over a wide range of radon concentrations and for time periods up to five days. It was concluded that the radon exposure system described provides a new analytic tool for the direct dosimetric evaluation of the toxic potential of radon gas and its emitted alpha particles.

CYTOGENETICS

19

Ellard S, Mohammed Y, Dogra S, Woelfel C, and others. THE USE OF GENETICALLY ENGINEERED V79 CHINESE HAMSTER CULTURES EXPRESSING RAT LIVER CYP1A1, 1A2 AND 2B1 cDNAs IN MICRONUCLEUS ASSAYS. *Mutagenesis* 1991;6(6):461-470.

A comparative study was undertaken of the bioactivation of a panel of promutagens by V79 Chinese hamster cells genetically engineered to metabolic competence. In vitro micronucleus assays of the test agents in V79 cultures in the presence of an Aroclor induced rat S9 yielded positive results. In the genetically engineered cell lines, benzo(a)pyrene was metabolized specifically by the 3-methylcholanthrene inducible rat liver CYP1A1 (cell line XEM2) whereas cyclophosphamide increased the micronucleus in phenobarbital inducible CYP2B1 (SD1). Exposure to tobacco particulate matter gave a greater induction of micronuclei in the XEM2 cell line compared to V79 cultures, implicating polycyclic aromatic hydrocarbons in addition to direct-acting compounds as causal agents in the genotoxicity of tobacco particulate matter. Other promutagens were also tested. The cytokinesis blocked in vitro micronucleus assay provides a faster, simpler alternative to metaphase analysis, and kinetochore labelling techniques that enables the discernment of both structural and numerical chromosome changes. The inclusion of metabolically competent test strains in the in vitro micronucleus assay therefore may create a powerful system for detecting genotoxins and may be extended to elucidate both mechanisms of bioactivation and modes of genotoxic insult.

20

Albertini S, Zimmermann FK. THE DETECTION OF CHEMICALLY INDUCED CHROMOSOMAL MALSEGREGATION IN SACCHAROMYCES

CEREVISIAE D61.M: A LITERATURE SURVEY (1984-1990).
Mutat Res 1991;258(3):237-258.

The objective of the study was to summarize published data obtained with a recently developed tester strain deemed suitable for the detection of chromosomal malsegregation in yeast. Results from 25 papers were reviewed in which numerical data for 111 chemicals tested in *Saccharomyces cerevisiae* D61.M are reported (a total of 316 independent tests; 279 acceptable, 37 not meeting predefined experimental criteria). Recommendation is made for standardization of methods and protocols for screening purposes. In addition, a comparison with in vitro tubulin assembly data using mammalian tubulin is presented.

CYTOTOXICITY

21

Duez P, Livaditis A, Guissou PI, Sawadogo M, Hanocq M.
USE OF AN AMOEBA PROTEUS MODEL FOR IN VITRO CYTOTOXICITY TESTING IN PHYTOCHEMICAL RESEARCH: APPLICATION TO EUPHORBIA HIRTA EXTRACTS. J Ethno Pharmacol 1991;34(2-3):235-246.
(24 REFS)

A low cost screening method using *Amoeba proteus* for the in vitro testing of the cytotoxic potential of plant extracts is described. Known anticancer drugs were tested using this method. The model was applied to the analysis of *Euphorbia hirta* aqueous extracts.

22

Patel BC M, 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 intact and membrane-damaged cells was developed. This procedure utilizes fluorescein diacetate and ethidium bromide as fluorescent probes. The properties of the probes and the collapse in the selective cytoplasmic membrane permeability barrier of the damaged cells ensure the principal feature of the test procedure, that 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 materials on the basis of cytotoxicity.

23

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):469-484.

The effect of a number of ceramic raw materials and airborne samples in the workplace 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 dusts causing various types of reactions in lungs. Dusts classified as "quartz type" in vitro were also cytotoxic in vivo. Dusts were also divided into three groups according to their degree of hazard, taking into

consideration the time before an inflammatory reaction is detected and the degree of inflammatory response as characterized by identified biochemical parameters. One feature of the test systems of practical significance is that in vitro experiments require only 15 mg of dust, while in vivo experiments require 100 mg.

24

Pissiotis E, Spangberg L SW. TOXICITY OF PULPISPAD USING FOUR DIFFERENT CELL TYPES. *Int Endod J* 1991; 24(5):249-257.

The cytotoxic effect of zinc oxide-eugenol-based paste (Pulpispad) was evaluated in vitro after setting for 1 day and 1 week. Target cells were L929 cells, gingival, periodontal ligament and pulpal fibroblasts. The material was incubated with the cells for 4 and 24 hours and its toxicity was evaluated with the ⁵¹Cr-release method. Pulpispad was highly cytotoxic to all cell lines even after setting for 1 week. The various responses among the four cell lines indicated that diploid cell lines can, under certain circumstances, be less sensitive than aneuploid cell lines. It is suggested that in the evaluation of biomaterials the choice of cell lines be carefully considered, as they can exhibit varying sensitivities.

25

Witzmann F, Bale S, London S. CELLULAR TOXICITY AS DETERMINED BY TWO-DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS IN VITRO TOXICOLOGY. *J Molec Cell Toxic* 1990;3(3):205-21.

The effect of perfluoro-n-decanoic-acid (PFDA) on mouse lymphoma cell line L5178Y TK(1) was examined to determine the utility of a two dimensional polyacrylamide gel electrophoretic analytical approach. High resolution two dimensional polyacrylamide gel electrophoresis was used to analyze the intracellular proteins and those appearing in the spent culture medium. A measurable cytotoxic effect was exerted by PFDA at concentrations as low as 2 micrograms/milliliter. As a result of PFDA exposure, gel patterns of numerous cellular proteins changed. In some cases the expression of specific polypeptides was diminished. In other cases it was abolished entirely. It was suggested that results support the notion that in vitro toxicity testing methods may serve as an important adjunct to traditional test systems with the inclusion of two dimensional electrophoresis.

26

Saito H, Iwami S, Shigeoka T. IN VITRO CYTOTOXICITY OF 45 PESTICIDES TO GOLDFISH GF-SCALE (GFS) CELLS. *Chemosphere* 1991;23(4):525-538.

The cytotoxicities of 45 pesticides including 18 organophosphates and 7 carbamates to GF-Scale (GFS) cells, derived from the scale of goldfish (*Carassius auratus*), were determined with the 24-hr neutral red assay. Carbamates were less cytotoxic (200 mg/L < NR50) than organophosphates. The 24-h NR50 values for the cytotoxicity to GFS cells were compared to published 48-hr LC50 values for the acute lethal toxicity of the same pesticides to carp. A significant correlation was found between the NR50 and LC50 values ($n = 34$, $r = 0.848$, $s = 0.381$, $F = 81.5$) excluding 11 pesticides for which neither NR50 nor LC50 values were not obtained because of their low solubility. Results indicated that the in vitro cytotoxicity assays using GFS cells are useful for the prediction of the acute toxicity of pesticides to fish.

27

Boue-Grabot M, Pinon JF, Halaviat B, Bernardin G, and others. AN NEW IN VITRO METHOD FOR TESTING THE CYTOTOXICITY OF NON-WATER-SOLUBLE SUBSTANCES IN CELL CULTURES. *Bull Soc Pharm Bordeaux* 1990;129(1-2-3-4):99-103.

Non-water-soluble substances (cosmetics and surfactants were used as test compounds) were dissolved in paraffin and allowed to contact cultured human skin fibroblasts in aqueous media. Some of the test substances would be taken up by the cells because of their oil/water

partition coefficient. The damage to cell viability was then determined by standard microscopy techniques. Even though the amount of hydrophobic substance in the hydrophilic cell medium may be small, the results still give an accurate index of the cytotoxicity of the substance.

28

Fautrel A, Chesne C, Guillouzo A, De Sousa G, Placidi M, Rahmani R, Braut F, Pichon J, Hoellinger H, et al. A MULTICENTER STUDY OF ACUTE IN VITRO CYTOTOXICITY IN RAT LIVER CELLS. *Toxicol in Vitro* 1991;5(5-6):543-7.

A group of 31 antibiotics, anticancer drugs, and solvents were incubated for 20 hours with normal rat hepatocytes and FaO hepatoma cells. Four cytotoxic endpoints were evaluated: the ratio of extracellular lactate dehydrogenase to total lactate dehydrogenase, total cellular protein content, reduction of a tetrazolium salt, and neutral red uptake. For each test, IC50 values were calculated. More compounds were cytotoxic to hepatocytes than to hepatoma cells. On the basis of the IC50 values, a few compounds were much less cytotoxic than predicted from in vivo data, suggesting that a simple experimental protocol and non-specific cytotoxicity parameters were not sufficient to test certain drug families. However, such methods define the concentration range of the drugs for further analysis using more specific tests.

29

Dive C, Gregory CD, Phipps DJ, Evans DL, Milner AE, Wyllie AH. ANALYSIS AND DISCRIMINATION OF NECROSIS AND APOPTOSIS (PROGRAMMED CELL DEATH) BY MULTIPARAMETER FLOW CYTOMETRY. *Biochim Biophys Acta* 1992;1133(3):275-85.

Necrosis and apoptosis are two distinct modes of cell death which differ in morphology, mechanism and incidence. Membrane disruptants, respiratory poisons and hypoxia cause ATP depletion, metabolic collapse, cell swelling and rupture leading to inflammation. These are typical features of necrosis. Apoptosis plays a crucial role in embryogenesis and development and is also prevalent in tumours. It is characterised by cell shrinkage, chromatin condensation and systematic DNA cleavage. In vivo, apoptosis is almost impossible to quantify due to problems of heterogeneity and the short half-life of an apoptotic cell. In vitro, mechanistic studies are further complicated by a late phase of apoptosis where the cell membrane becomes permeable to vital dyes and which occurs in the absence of phagocytes. A novel and rapid

multiparameter flow cytometric assay is described which discriminates and quantifies viable, apoptotic and necrotic cells via measurement of forward and side light scatter and the DNA-binding fluorophores Hoechst 33342 and propidium. It is anticipated that mechanistic studies of apoptosis in a variety of cell types will greatly benefit from this mode of analysis.

30

Armstrong MJ, Bean CL, Galloway SM. A QUANTITATIVE ASSESSMENT OF THE CYTOTOXICITY ASSOCIATED WITH CHROMOSOMAL ABERRATION DETECTION IN CHINESE HAMSTER OVARY CELLS. *Mutat Res* 1992;265(1):45-60.

Regulatory guidelines suggest testing chemicals up to cytotoxic doses in chromosomal-aberration assays. To investigate the utility and limitations of various cytotoxicity indicators Chinese hamster ovary (CHO) cells were used to test 8 chemicals with differing ratios of cytotoxicity to clastogenicity. Measured were immediate or delayed cell killing and growth inhibition (ATP levels, cell counts, colony-forming efficiency, CFE) and cell-cycle perturbations (mitotic index, MI; average generation time, AGT). Aberrations (ABS) were scored 10 and 24 h from the beginning of the 3-h treatment. All 8 compounds induced ABS at concentrations that reduced cell growth at 24 h by 50% or less. Cells with multiple ABS were seen at 24 but not at 10 h, and often confirmed clastogenicity when there was only a weak increase in the percentage of cells with aberrations. MI suppression often did not correlate with average generation time (AGT). By 24 hours the MI for all chemicals had recovered, sometimes exceeding control levels. The MI has limited value for dose selection. In conclusion, even weakly active chemicals were detected without exceeding a 50% growth reduction by 24 hours.

31

Morita H, Umeda M, Ogawa HI. MUTAGENICITY OF VARIOUS CHEMICALS INCLUDING NICKEL AND COBALT COMPOUNDS IN CULTURED MOUSE FM3A CELLS. *Mutat Res* 1991;261(2):131-137. (24 REFS)

The cytotoxic and mutagenic effects of various chemical compounds were evaluated in suspension cultures of FM3A cells. The FM3A cells, a CSH-mouse mammary carcinoma line, were treated with various concentrations of: N-methyl-N'-nitro-N-nitrosoguanidine, N-ethyl-N'-nitro-N-nitrosoguanidine, sterigmatocystin, mitomycin-C, Trp-P-1, Trp-P-2, nickel(II)-chloride, nickel(II)-acetate,

cobalt(II)-chloride, and complexes of nickel and cobalt and they were also exposed to X-rays. After the cells were treated with the test compounds for 3 to 48 hours, they were washed and cultured for determination of mutation expression and survival. For metabolic activation assessment, cells were exposed to a test compound for 5 hours in a reaction mixture consisting of rat liver S15, then subjected to the mutation assay. X-Ray irradiation was performed with a linear accelerator and cells were immediately assayed for cell survival and subjected to the mutation assay. Nickel and cobalt compounds, nickel complex, cobalt-chloride, and cobalt complex were cytotoxic to FM3A cells. Compared to other compounds, some nickel and cobalt compounds were weakly mutagenic.

32

Bean CL, Galloway SM, Bradley MO. EVALUATION OF THREE IN VITRO ASSAYS FOR ASSESSMENT OF MEMBRANE DAMAGE BY SURFACTANTS. *In Vitro Toxicol, J Molec Cell Toxicol* 1991; 4(2):133-144. (17 REFS)

Three genotoxicity in-vitro based assays, performed under standard conditions were evaluated for ability to predict surfactant activity (a cytotoxic mechanism associated with lysis of cell membranes). The assays used: 6-carboxyfluorescein dye leakage from liposomes; hemoglobin release from dog red blood cells; and dye leakage from the ghosts of dog red blood cells. Three common detergents were used as test materials in the evaluation: Triton-X-100, sodium lauryl sulfate (SLS), and cetyltrimethyl-ammonium bromide (CTAB). Assay results were compared to the survival of Chinese-hamster-ovary (CHO) cells after treatment. The most sensitive assay was for ghost dye leakage with median effective concentrations values equivalent to or lower than those of the CHO cell counts. It was concluded that the red cell lysis or the red ghost dye leakage assays would facilitate determination of surfactant activity in a compound within the dose range that causes cytotoxicity in standard genotoxicity assays.

33

Laschinski G, Vogel R, Spielmann H. CYTOTOXICITY TEST USING BLASTOCYST-DERIVED EUPLOID EMBRYONAL STEM CELLS: A NEW APPROACH TO IN VITRO TERATOGENESIS SCREENING. *Reprod Toxic* 1991;5(1):57-64. (23 REFS)

Described was a study of the cytotoxic effects of selected xenobiotics on embryonal stem cells (ESC) using well established methods such as kenacid-blue,

neutral-red, and dimethylthiazol-diphenyl tetrazolium-bromide was presented. Since ESC grow very rapidly and show signs of active metabolism, their sensitivity to toxic agents may be different from that of differentiated cells and may offer a new screening system for detection of teratogenic effects in-vitro. ESC showed a higher sensitivity to known teratogens than fibroblast cultures. Although some xenobiotics were classified as false negatives in the ESC system it was concluded that ESC cytotoxicity assays holds promise for future screening work. Among antineoplastic agents, comparison of the dose response curves of ESC and fibroblasts allows for differentiation between three characteristic groups of such agents.

34

Fishwild DM, Staskawicz MO, Wu HM, Carroll SF.
CYTOTOXICITY AGAINST HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS AND T CELL LINES MEDIATED BY ANTI-T CELL IMMUNOTOXINS IN THE ABSENCE OF ADDED POTENTIATOR. Clin Exp Immunol 1991;86(3):506-13.

Several in vitro assays have indicated that anti-T cell immunotoxins (IT), composed of monoclonal antibodies (MoAbs) conjugated to ricin A chain (RTA), are maximally effective against T cells only in the

presence of potentiators. It was thought that such IT might not be sufficiently cytotoxic to deplete T cells in vivo upon administration to patients. Therefore, the authors re-evaluated the in vitro assays. It was found that T cell lines may not serve as accurate models to determine the efficacy of IT against peripheral blood mononuclear cells (PBMC) in vitro or in vivo. In addition, IT-induced cytotoxicity of PBMC can be demonstrated in vitro at pharmacologically achievable concentrations in the absence of added potentiators.

DERMAL TOXICITY

35

Triglia D, Braa SS, Yonan C, Naughton GK.
IN VIRTO TOXICITY OF VARIOUS CLASSES OF TEST AGENTS USING THE NEUTRAL RED ASSAY ON A HUMAN THREE-DIMENSIONAL PHYSIOLOGIC SKIN MODEL. In Vitro Cell Develop Biol 1991; 27A(3):239-244. (28 REFS)

Toxicity data were presented for the following classes of test agents: 15 detergents, five alcohols, ten metal chlorides, five perfumes and colognes, four shampoos,

three conditioners, three moisturizers, three pesticides, and four antimicrobial preservatives. Dose dependent cytotoxic responses were observed for all test agents in each category. A new three dimensional human skin model consisting of several layers of actively dividing and metabolically active human neonatal foreskin derived fibroblasts and epidermal keratinocytes grown on nylon mesh were used to assess the toxicity of these agents. A slight modification of the published neutral red viability assay was used for endpoint determination. The potential utility of the human dermal fibroblast epidermal keratinocyte, three dimensional substrate as an in-vitro toxicology assay system was described.

36

Osborne R, Perkins MA. IN VITRO SKIN IRRITATION TESTING WITH HUMAN SKIN CELL CULTURES. *Toxicol in Vitro* 1991; 5(5-6):563-7.

Cultured human skin cells are a potentially useful model for skin irritancy testing. The effects of chemical irritants were evaluated on human epidermal keratinocytes (NHEK) and on keratinocyte-dermal fibroblast (NHEK/DF) cocultures. Cell viability in NHEK cultures, measured as incorporation of the vital dye Neutral Red (NR), was reduced in a dose-dependent manner in response to the chemical irritants tested. The half-maximal effective concn. (NR50) values correlated with irritation scores in human patch tests with these materials. Certain materials were incompatible with this test system. NHEK/DF cultures were treated with ten prototype surfactants. Close correlation was obtained for the dose-response characteristics of all the endpoints tested, and between the in vitro responses and human patch test scores for the surfactants tested. Results demonstrate the usefulness of human skin cell cultures and of cell viability, cytotoxicity, and inflammatory mediator release as endpoints, for the in vitro assessment of skin irritancy.

DEVELOPMENTAL TOXICITY

37

Rayburn JR, DeYoung DJ, Bantle JA, Fort DJ, McNew R. ALTERED DEVELOPMENTAL TOXICITY CAUSED BY THREE CARRIER SOLVENTS. *J Appl Toxic* 1991;11(4):253-260.

Experiments were conducted to determine whether carrier solvents interacted with teratogens such as methylmercury-chloride (MMC) and trichloroethylene (TCE) to affect survival, development and growth of *Xenopus*

embryos. Three different endpoints (mortality, malformation, and embryo growth) were assessed using the Frog Embryo Teratogenesis Assay. Solvents used included dimethylsulfoxide (DMSO), acetone, and triethylene-glycol (TG). The lethal effect of both teratogens was potentiated by DMSO but this solvent did not alter significantly the rate of malformation. Mortality for both teratogens was also increased by acetone, but acetone only increased the MMC malformation greater than the additive effects. TG increased the mortality and malformations caused by TCE. Additive effects were noted for growth for all solvents with the teratogens. The authors urged that the choice of carrier be made with caution.

EMBRYOTOXICITY

38

Dawson DA. ADDITIVE INCIDENCE OF DEVELOPMENTAL MALFORMATION FOR XENOPUS EMBRYOS EXPOSED TO A MIXTURE OF TEN ALIPHATIC CARBOXYLIC ACIDS. *Teratology* 1991; 44(5):531-546. (28 REFS)

A report was conducted to determine the effect of a mixture of ten aliphatic acids on embryonic development. The acids tested included valproic acid, hexanoic acid, octanoic acid, butyric acid, 2-ethylhexanoic acid, pentanoic acid, propionic acid, 2-methylpentanoic acid, heptanoic acid, 3-methylbutyric acid, and a mixture of these compounds. A modified Frog Embryo Teratogenesis Assay, the Xenopus (FETAX) protocol, was used. The concentration additive rate of malformation indicated that all ten acids were likely to induce malformation in Xenopus embryos in a similar manner. Quantitative structure activity relationship analysis revealed that developmental malformation induced by the acids was highly correlated with hydrophobicity and molar refractivity. It was suggested that this approach had potential application in determining compounds that induce developmental malformations in a similar manner, when metabolism and pharmacokinetic factors are considered.

39

Oglesby LA, Ebron-McCoy MT, Logsdon TR, Copeland F, Beyer PE, Kavlock RJ. IN VITRO EMBRYOTOXICITY OF A SERIES OF PARA-SUBSTITUTED PHENOLS: STRUCTURE, ACTIVITY, AND CORRELATION WITH IN VIVO DATA. *Teratology* 1992; 45(1):11-34.

The embryotoxicity of phenol and twelve para-substituted congeners on mid-gestation rat embryo was evaluated in vitro. Through application of correlative procedures and

stepwise regression, equations describing the relationship between physical-chemical properties and various measures of activity were developed. Embryotoxicity was quantified by the log of the reciprocal of the potency estimates for reduction in selected growth parameters and induction of four morphological defects. In general, co-cultured hepatocytes ameliorated embryotoxicity; only phenol-induced embryotoxicity was enhanced by the presence of hepatocytes. In the absence of hepatocytes, measures of growth retardation were positively correlated with molar refractivity of the phenols. With hepatocytes, lipophilicity became important for describing the potential to induce growth deficits. Data from preliminary dosimetry studies suggest that phenol congeners may accumulate in embryos exposed in vitro more readily than with in vivo exposures. Potency calculations based on such dosimetry information may demonstrate better correlations between data and allow additional relationships between chemical structure and activity to be developed.

40

Anon. ALTERNATIVE APPROACHES FOR THE ASSESSMENT OF REPRODUCTIVE TOXICITY (WITH EMPHASIS ON EMBRYOTOXICITY/TERATOGENICITY). European Chemical Industry Ecology and Toxicology Centre, Avenue Louis 250, Bte 63, 1050 Bruxelles, Belgium, Nov. 1...1990.

This monograph reviews the present status of alternative in vivo and in vitro tests developed for evaluating the effects of chemicals on reproduction, and assesses their relevance and validity. It was concluded that at present, no test can totally replace the existing reproductive toxicity tests which use live animals. In many instances alternative methods can be used to screen chemicals for further technical development or further testing, and to provide information on the reproductive toxicity of those chemicals which do not warrant full-scale testing. Application of the results from the alternative test approaches to human hazard assessment was also discussed.

41

Tsuchiya T, Burgin H, Tsuchiya M, Winternitz P, Kistler A. EMBRYOLETHALITY OF NEW HERBICIDES IS NOT DETECTED BY THE MICROMASS TERATOGEN TESTS. Archives of Toxicology 1991;65(2):145-149.

New herbicides were evaluated in-vivo and in micromass culture to determine the validity of in-vitro techniques for evaluating possible embryo-lethal and teratogenic effects. Fifteen herbicides were given orally to female

FU-albino-rats from day six through 15 of gestation, and embryos were evaluated for signs of teratogenicity and embryoletality. The herbicides were embryoletal but not teratogenic at dose ranges of 0.2 to 200mg/kg. To compare in-vivo to in-vitro techniques, primary micromass culture of day 13 rat embryo limb/bud and midbrain cells were incubated with the various herbicides for 7 days. Experimental results indicated that there was no correlation between in-vivo and in-vitro data with respect to embryoletality. To determine if cytotoxicity was reflective of embryoletality, IC50 values for colony formation were determined from Chinese-hamster-V79-cells in the presence or absence of S9 liver fraction. Results showed that there was some correlation between cytotoxic effects with herbicides. It was concluded that micromass culture was not useful for predicting embryoletality, but inhibition of colony formation of V79 cells is indicative of embryoletality.

GENOTOXICITY

42

Benigni R, Giuliani A. WHAT INDICATION IS COMMON TO DIFFERENT GENOTOXICITY DATA BASES? *Mutat Res* 1991; 253(2):115-122.

This paper studies the relationship among 4 in vitro assays: Salmonella mutation (STY), mouse lymphoma L5178Y cell mutation (MLY), chromosomal aberrations in CHO cells (CHA), and sister-chromatid exchanges in CHO cells (SCE), in 3 different data bases: U.S. National Toxicology Program (NTP), International Program for the Evaluation of Short-term Tests for Carcinogens (IPESTTC), and International Program on Chemical Safety (IPCS). The analysis was performed by using factor analysis to model each data base. With this tool, it was possible to separate different elements (or components) which play a role in each data base. It has also been possible to demonstrate that - together with some specificities of the data bases - there is a common effect which is independent of the data bases, and which typically represents the 'true' relationship among the assays. The implication of the results is 2-fold. First, it is extremely reassuring that the 3 most important comparative studies agree and show common evidence, and that this can be recognized rationally. Secondly, this evidence implies that the scientists involved in mutagenicity research must face the task of exploring and explaining such relationships.

43

Kramers P GN, Knaap A G AC, Van Der Heijden CA, Taalman R D FM, Mohn GR. ROLE OF GENOTOXICITY ASSAYS IN THE

REGULATION OF CHEMICALS IN THE NETHERLANDS: CONSIDERATIONS AND EXPERIENCES. *Mutagenesis* 1991; 6(6):487-494.

Discussed is genotoxicity testing and data interpretation as applied in The Netherlands in the context of the regulation of chemicals. Guidelines for such testing were first formulated in 1981 and evolved in practice, based on 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 always been cornerstone of the Dutch approach. Examples are given of how short-term tests contributed to the toxicological evaluation of chemicals in The Netherlands.

44

Oshiro Y, Piper CE, Balwierz PS, Soelter SG. CHINESE HAMSTER OVARY CELL ASSAYS FOR MUTATION AND CHROMOSOME DAMAGE: DATA FROM NON-CARCINOGENS. *J Appl Toxicol* 1991; 11(3):167-177.

The genotoxic potential of some noncarcinogens was examined in Chinese-hamster ovary (CHO) cell assays. It was concluded that the HGPRT gene mutation assay appears to parallel the negative rodent carcinogenicity bioassay results with the chemicals tested. The micronucleus assay, although it is a more sensitive indicator of in vitro genotoxicity, may cause more false positives when used to screen suspected carcinogens.

45

Scott D, Galloway SM, Marshall RR, Ishidate M Jr, and others. GENOTOXICITY UNDER EXTREME CULTURE CONDITIONS. A REPORT FROM ICPEMC TASK GROUP 9. *Mutat Res* 1991; 257(2):147-20.

The influence of extreme culture conditions upon the results of in-vitro genotoxicity assays and their relevancy to in-vivo studies were discussed. Extreme culture conditions discussed included excessively high concentrations of test agents, high levels of cytotoxicity, the use of metabolic activation systems, and extremes of pH. Many studies indicate that when rodent models have been used to predict human genotoxicity, rodent genotoxicants are detected in in vitro systems in which concentrations that are highly cytotoxic are used. It has not been possible to define an upper limit for cytotoxicity in in vitro tests other than on a case by case basis. Assays that utilize

metabolic activation systems derived from isolated microsomes appear to be capable of generating toxic and mutagenic species. The use of nonphysiological pHs in in vitro test systems can not only affect the mutagenic response of the test agent but can be mutagenic in their own right. It was concluded that the artefactual genotoxicity associated with the use of extreme culture conditions does not explain all of the discrepancy between in vitro and in vivo results.

46

Negishi T, Shiotani T, Funjikawa K, Hayatsu H. THE GENOTOXICITIES OF N-NITROSAMINES IN DROSOPHILA MELANOGASTER IN VIVO: THE CORRELATION OF MUTAGENICITY IN THE WING SPOT TEST WITH THE DNA DAMAGES DETECTED BY THE DNA-REPAIR TEST. *Mutat Res* 1991;252(2):119-128.

The genotoxic activities of N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosodi-n-butylamine (NDBA), N-nitrosomorpholine (NMOR), N-nitroso-piperidine (NPIP), N-nitrosopyrrolidine (NPYR), N-nitrosodiphenylamine (NDPhA), N-nitrosoproline (NPRO), and N-nitrosothiopropine (NTPRO) were studied in *Drosophila-melanogaster*. NDMA, NDEA, NDBA, NMOR, NPYR, and NPIP were known carcinogens. NPRO was noncarcinogenic. NDPhA had given equivocal results in rodent carcinogenicity tests. Genotoxic activity was assessed in the wing hair spot test and by a DNA repair assay based on the survival of Rec(-) males relative to survival of Rec(+) females. All compounds which gave positive results were also tested in the Ames/Salmonella assay using strain (TA-100) and S9 activation. It was concluded that NDMA, NDEA, NDBA, NMOR, NPIP, and NPYR are genotoxic in both *Drosophila* assays. *Drosophila* tests may be useful for screening suspected carcinogens. The DNA repair test warrants further development as it is simpler than the wing spot test.

47

Godin CS, Myhr C, Lawlor TE, Young RR, Murli H. GENOTOXICITY ASSESSMENT OF CHLOROTRIFLUOROETHYLENE TETRAMER ACID USING A BATTERY OF IN VITRO AND IN VIVO/IN VITRO ASSAYS. *Govt Reports Announcements & Index (GRA&I)*, Issue 01, 1992.

Chlorotrifluoroethylene (CTFE) tetramer acid, the perhalogenated fatty acid metabolite of the eight-carbon oligomer of polychlorotrifluoroethylene (pCTFE), was evaluated in in vitro bioassays to assess

its potential genotoxic activity. The assays conducted were the Ames Salmonella/microsomal mutagenicity assay, the Chinese hamster ovary (CHO) gene mutation assay, the CHO/sister chromatid exchange (SCE) and chromosome aberration assays, the BALB/c-3T3 cell transformation assay and in vivo/in vitro unscheduled DNA (UDS) and S-phase synthesis assays. CTFE tetramer acid did not demonstrate genotoxic potential in any of the in vitro assays and was also negative in the UDS assay. Based upon the results of the test battery no genetic risk from exposure to CTFE could be predicted.

48

Legator MS, Ward JB Jr. USE OF IN VIVO GENETIC TOXICITY DATA FOR RISK ASSESSMENT. *Mutat Res* 1991;250(1-2)457-65.

The authors propose the use of a multiple end-point in vivo comprehensive testing protocol using rodents for genetic toxicity evaluation, especially for genotoxic carcinogens not detected by in vitro methods. Studies using subacute exposure to low levels of test agents by routes consistent with human exposure can be a useful adjunct to some in vitro methods currently used to provide data for risk assessment. Evaluations can include metabolic and pharmacokinetic endpoints, in addition to genetic toxicity studies, in order to provide a comprehensive examination of the mechanism of toxicity of the agent. A categorical risk assessment procedure can be used which would consider, in order of priority, genetic damage in man, genetic damage in animals that is highly relevant to disease outcome (mutation, chromosome damage), and data from animals that is of less certain relevance to disease. Action levels of environmental exposure would be determined based on the lowest observed effect of levels or the highest observed no effect levels, using subacute low level exposure studies in rodents. If genetic toxicity is to achieve a prominent role in evaluating carcinogens and in characterizing germ-cell mutagens, minimal testing requirements must be established to ascertain the risks associated with environmental mutagen exposure. The use of the in vivo approach described here should provide the information needed to meet this goal. In addition, it should allow truly epigenetic or nongenotoxic carcinogens to be distinguished from the genotoxic carcinogens that are not detected by in vitro methods.

49

Gao N, Aidoo A, Heflich RH. ANALYSIS OF RAT LYMPHOCYTE

ACTIVATION OF BENZO[A]PYRENE, 2- ACETYLAMINOFLUORENE, AND SEVERAL OF THEIR METABOLITES TO MUTAGENIC AND DNA-DAMAGING SPECIES IN VITRO. Teratog Carcinog Mutagen 1991;1(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 genotoxic species. 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. Human lymphocytes, but not rat lymphocytes, mediated a positive mutagenic response with BaP 7,8-dihydrodiol in Chinese hamster ovary UV5 cells. Although rat lymphocytes may metabolize certain proximal genotoxic chemicals to DNA-damaging species (e.g., N-hydroxy-2-AAF), these 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 use of the rat model to predict the genotoxicity of chemicals in humans.

50

Mackay JM, Elliott BM. DOSE-RANGING AND DOSE-SETTING FOR IN VIVO GENETIC TOXICOLOGY STUDIES. Mutat Res 1992, Feb;271(1):97-9.

No abstract.

51

Helen B, Singh I, Sandhu SS, Doehmer J, Athwal RS. A HYBRID CELL LINE EXPRESSING CYTOCHROME P450 GENES APPLICATION IN THE DETECTION OF GENOTOXIC EFFECTS OF CHEMICALS. PROCEEDINGS OF THE 8TH INTERNATIONAL CONGRESS OF HUMAN GENETICS, WASHINGTON, D.C., USA, OCTOBER 6-11, 1991. Am J Hum Genet 1991;49(4):448.

No abstract.

52

Su TC, Elder FB. MAMMALIAN CELLS FOR TESTING GENOTOXICITY OF CHEMICAL MUTAGENS IN ADOLPH, K. W. (ED.). ADVANCED TECHNIQUES IN CHROMOSOME RESEARCH. XIII . MARCEL DEKKER, INC.: NEW YORK, NEW YORK, USA; BASEL, SWITZERLAND, 1991; 0(0):391-410.

No abstract.

53

Gasiorowski K, Szyba K, Sawicka J, Osada A, Gonera B, Gulanowski B. GENOTOXICITY OF AIR POLLUTION IN A NON-FERROUS METAL FOUNDRY IN THREE BACTERIAL TESTS AND A MICRONUCLEUS TEST A COMPARATIVE STUDY. Polish Journal of Occupational Medicine 1989;2(2):161-170. (16 REFS)

The genotoxicity of dusts and fumes in a nonferrous metal foundry was investigated. Dust and fume samples were collected at six sites in a Polish nonferrous metal foundry. They were extracted with acetone. The extracts were tested for genotoxicity in the Ames/Salmonella assay using strain (TA-98) with or without rat liver S9 activation, the umu SOS assay in Salmonella-typhimurium strain (TA-1535/psK1002), the Bacillus-subtilis H12-rec⁺rec(-) repair assay, and the bone marrow micronucleus assay in male Balb/c-mice. Doses used in the in-vitro assays ranged from 200 to 800 micrograms per plate or from 200 to 800micrograms/disc. All samples were mutagenic in the Ames/Salmonella assay with or without S9 activation. The mutagenic activity in the extracts was dose dependent. In the umu test, the 200micrograms/plate dose produced negative results in all samples. All doses produced positive responses in the bacterial repair test. Most doses (0.156 to 50.0mg/kg) produced positive responses in the mouse bone marrow micronucleus test; however, responses were not dose dependent. It was concluded that the Ames/Salmonella assay is the most useful for monitoring genotoxicity of pollutants in the workplace air of nonferrous metal foundries.

54

von der Hude W, Carstensen S, Obe G. STRUCTURE-ACTIVITY RELATIONSHIPS OF EPOXIDES: INDUCTION OF SISTER-CHROMATID EXCHANGES IN CHINESE HAMSTER V79 CELLS. Mutat Res 1991; 249(1):55-70. (20 REFS)

In an effort to investigate structure/activity relationships for genotoxic damage caused by epoxides in mammalian cells, the sister chromatid exchange (SCE) inducing potencies in Chinese-hamster-V79 cells were determined for 58 epoxides. The test system allowed for rapid screening of many substances and gave reproducible results. In general results regarding the structure/activity relationship corresponded well with results obtained in the

Ames test. The monosubstituted epoxides had the highest mutagenic activity. Within this group electron withdrawing properties of substituents elevated the genotoxicity, as noted with the halogenated epoxides and the phenylglycidylethers. Electron donating properties generally reduced the genotoxic properties as in the case of the glycidylethers. Unexpected and contradictory results compared with the bacterial Ames test were obtained for the olephenic epoxides. The linear diepoxides were the most potent SCE inducers of the epoxides tested. It was concluded that the V79/SCE test is a good tool to investigate quantitative structure/activity relationships with mammalian cells in-vitro.

55

DeMarini DM, Lawrence BK, Brooks HG, Houk VS.
COMPATIBILITY OF ORGANIC SOLVENTS WITH THE MICROSCREEN
PROPHAGE-INDUCTION ASSAY: SOLVENT-MUTAGEN INTERACTIONS.
Mutat Res 1991;263(2):107-113. (22 REFS)

A study was made of the compatibility of various organic solvents with the Microscreen prophage induction assay for genotoxicity and of mutagen solvent interactions in this system. Solvents tested included acetone, benzene, chloroform, dimethyl sulfoxide (DMS), ethanol, n-hexane, isopropanol, methanol, methylene chloride, toluene and a mixture of isomers of xylene. Effects of acetone, DMS and methanol on genotoxicity of 2-nitrofluorene (NF) and 2-aminoanthracene (AA) were determined. The Microscreen assay measured induction of the prophage lambda lysogen WP2s (lambda) in Escherichia-coli. Two fold dilutions of solvents from maxima of 10% or less were tested with or without male Sprague-Dawley rat liver S9 mix for metabolic activation. Prophage induction was not observed in the presence or absence of S9 with acetone, benzene, chloroform, ethanol, n-hexane, isopropanol, methanol, toluene or xylene even up to toxic concentrations. Acetone (with or without S9) and n-hexane (with S9) were not toxic up to the maximum concentration of 10%. Two samples of DMS were strong prophage inducers with or without metabolic activation. It was concluded that solvent selection and concentrations for prophage induction and other mutagen assays require careful consideration, and acetone is the preferred solvent for the Microscreen assay.

56

Whong WZ. DEVELOPMENT OF A LUNG-CELL MODEL FOR
STUDYING WORKPLACE GENOTOXICANTS. [Final Report]

Microbiology Section, LIB, DRDS, NIOSH, Cincinnati, Ohio, 23pages, 1990-1. (16 REFS)

The objectives of this 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. Results indicated that the best enzymatic separation of rat lung cells was a combined treatment of lung with 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 Micronucleus formation MN analyses. Both alveolar macrophages and primary lung cells could be used for in-vivo and in-vitro unscheduled DNA synthesis (UDS) assays. Primary lung cells in cultures retained their metabolic activation and DNA repair abilities. It was concluded that the lung cell genotoxicity assay system with different genetic endpoints may have potential usefulness for studying the pulmonary effect of environmental and occupational genotoxicants.

57

Ellard S, Mohammed Y, Dogra S, Wolfel C, Doehmer J, Parry JM. THE USE OF GENETICALLY ENGINEERED V79 CHINESE HAMSTER CULTURES EXPRESSING RAT LIVER CYP1A1, 1A2 AND 2B1 cDNAs IN MICRONUCLEUS ASSAYS. *Mutagenesis* 1991;6(6):461-70.

A comparative study involved the bioactivation of a panel of promutagens by V79 Chinese hamster cells genetically engineered to metabolic competence. In vitro micronucleus assays of the test agents in V79 cultures in the presence of an Aroclor induced rat S9 yielded positive results. In the genetically engineered cell lines, benzo[a]pyrene was metabolized specifically by the 3-methylcholanthrene inducible rat liver CYP1A1 (cell line XEM2), whereas cyclophosphamide increased the micronucleus frequency only in cultures expressing the phenobarbital inducible CYP2B1 (SD1). Upon exposure to tobacco particulate matter, a greater induction of micronuclei was observed in the XEM2 cell line compared to V79 cultures, implicating polycyclic aromatic hydrocarbons in addition to direct-acting compounds as causal agents in the genotoxicity of tobacco particulate matter. The cytokinesis blocked in vitro micronucleus assay provides a faster, simpler alternative to metaphase analysis, and kinetochore labeling techniques enable the discernment of both structural and numerical chromosome changes. The inclusion of metabolically competent test strains in the in vitro micronucleus assay

therefore creates a powerful system for detecting genotoxins and may be extended to elucidate both mechanisms of bioactivation and modes of genotoxic insult.

HEMATOTOXICOLOGY

58

Deldar A, Stevens CE, Rodocker KB. CANINE BFU-e PORGENITORS: ADAPTATION OF A REPRODUCIBLE ASSAY AND ANATOMICAL DISTRIBUTION. *Int J Cell Cloning* 1992; 9(6):579-93.

In vitro cloning assays are used increasingly in investigative hematotoxicology and in screening candidate compounds for their hematotoxic potential. To attempt to expand these applications, a practical cloning assay for erythroid burst-forming units (BFU-e) that uses a microplasma clot (MPC) system was adapted to the dog, a species used extensively in experimental hematology and drug development. This system offers advantage over the methylcellulose and soft agar culture systems by allowing specimen fixation and, therefore, morphological and cytochemical evaluation. The relative concentration of BFU-e was comparable among samples collected from the iliac crest, femur, and humerus. Serial cultures performed on individual dogs were highly reproducible and little variation in BFU-e activity was identified among dogs of comparable age. It was concluded that the MPC system is a practical and reproducible cloning system for early (BFU-e), as well as late erythroid colony-forming units (CFU-e) in the

dog. The concentration of BFU-e appears comparable throughout the active marrow; therefore, various anatomic sites can be used interchangeably for serial quantitative analysis of this progenitor.

HEPATOTOXICITY

59

Wormser U, Ben-Zakine S. THE MOUSE LIVER SLICE SYSTEM AS AN IN VITRO MODEL FOR TESTING THE PROTECTIVE EFFECT OF N-ACETYLCYSTEINE AGAINST HEPATOTOXIC AGENTS. *Toxicol Methods* 1991;1(1):66-74.

An in vitro liver slice system was used to test the antidotal effect of N-acetylcysteine (NAC) against hepatotoxic agents. The liver slice system has been used to test NAC antidotal activity against other hepatotoxins known to be metabolized to reactive radicals, such as carbon tetrachloride (CCl₄), bromobenzene, and furosemide. The hepatocellular

damage caused by these agents was slightly reduced by the NAC sulfhydryl agent. These findings may demonstrate the ability of the in vitro liver slice system to serve as a tool for primary assessment of the activity of various antidotes against toxic agents.

60

Fisher R, Barr J, Zukoski CF, Putnam CW, and others.
IN VITRO HEPATOTOXICITY OF THREE DICHLOROBENZENE ISOMERS IN HUMAN LIVER SLICES. Hum Exp Toxicol 1991;10(5):357-364.

The cytotoxicity of dichlorobenzenes in cultured rat liver slices has previously been shown to be strain specific and biotransformation related. In order to extrapolate animal models to humans, the dichlorobenzenes were incubated with human liver slices as a means to clarify their potential for hepatotoxicity in man. The degree of hepatotoxicity observed with the dichlorobenzenes depended on whether Waymouth's or Krebs-Henseleit was used as the incubation medium. All three dichlorobenzenes produced no significant differences from control when incubated in Waymouth's medium. However, in the Krebs-Henseleit buffer there was a substantial increase in cytotoxicity. The use of human liver tissues to evaluate potential toxicants merits consideration since the hepatotoxicity of xenobiotics and drugs in man is the ultimate question.

IMMUNOTOXICITY

61

Snyder CA, Valle CD. IMMUNE FUNCTION ASSAYS AS INDICATORS OF CHROMATE EXPOSURE. Environ Health Perspectives 1991;92:83-86.

The potential immunomodulatory effects of chromium were investigated through use of a series of in-vitro and in-vivo studies. Potassium-chromate was added in-vitro to T-lymphocyte and B-lymphocyte mitogen cultures. T-Lymphocyte responses were inhibited at all concentrations tested. B-Lymphocyte response was inhibited at all but the lowest concentration tested. Enhanced responses to T-lymphocyte and B-lymphocyte mitogens were noted among splenocytes isolated from rats exposed to potassium-chromate in drinking water. The addition of 0.1mg/l of chromium to a mixed lymphocyte culture containing splenocytes taken from chromium exposed rats increased five times the uptake of thymidine by these cells. The authors suggest that the increased responses of cells from rats exposed to chromium

indicate chromium induced sensitization and may be useful as a biological marker for chromium exposure.

LUNGS

62

Hu T, Xu G, Xie G, Cang S, Wang W. IN VITRO STUDY ON THE MEMBRANE PERMIABILITY OF ALVEOLAR MACROPHAGES BY USING LACTATE DEHYDROGENASE RELEASE ASSAY. Zhongguo Huanjing Kexue 1990;10(6):470-3.

An in vitro study on the condition of the membrane permeability of alveolar macrophages was carried out by using the lactate dehydrogenase (LDH) release assay for detecting cytotoxicity activity. Macrophage membrane could be injured by some industrial dusts or chemicals. The more cell membranes were damaged, the more LDH released into the medium. The assay is apparently useful, as well as sensitive for estimating cell membrane permeability.

63

Lee J, Akley NJ, Krunkosky TM, Adler KB. METHODS TO STUDY INTERACTIONS BETWEEN INHALED SUBSTANCES AND EPITHELIUM OF THE RESPIRATORY TRACT IN VITRO. Microbeam Anal 1990;25th:457-8.

The proposed air/liquid interface airway cell culture system allows for measurement of subtle alterations following interactions of airway epithelium with sublytic concentrations of particulate or gaseous materials that might be inhaled directly, or to inflammatory mediators (e.g., oxygen free radicals) known to be generated after inhalation of a number of potentially harmful materials. In conjunction with data regarding deposition patterns and observable morphological effects on pulmonary cells, studies on subtle ultrastructural and biochemical alterations in epithelial cells in vitro may be invaluable in elucidating intracellular mechanisms governing toxic or subtoxic responses to inhaled materials that may relate to subsequent pathological alterations.

METABOLISM

64

Mills LJ, Robson DL, Malcolm AR. INTERACTIVE EFFECTS OF ALDRIN, CYCLOHEXYLAMINE, 2,4-DIAMINOTOLUENE AND TWO PHORBOL ESTERS ON METABOLIC COOPERATION BETWEEN V79 CELLS. Carcinogenesis 1991;12(7):1293-1299. (60 REFS)

The interactive effects of aldrin, cyclohexylamine,

2,4-diaminotoluene (DAT), phorbol-12-myristate-13-acetate (PMA), and phorbol-12,13-dibutyrate (PDB) on metabolic cooperation were studied in-vitro. Aldrin, cyclohexylamine, DAT, PMA, and PDB were tested for their effects on intercellular communication in the Chinese-hamster-V79 lung fibroblast metabolic cooperation assay alone or in binary mixture. Recovery of 6-thioguanine resistant mutants at the HGPRT locus was used as the endpoint in the V79 assay. The degree of metabolic cooperation was inversely related to the number of viable mutant cells. It was concluded that chemicals in mixtures may interact to yield responses different from those expected on the basis of individual chemical exposures. Interactive effects must be considered when performing the V79 cell metabolic cooperation assay on mixtures.

METHODOLOGY

65

Snyder CA, Valle CD. LYMPHOCYTE PROLIFERATION ASSAYS AS POTENTIAL BIOMARKERS FOR TOXICANT EXPOSURES. *J Toxicol Environ Health* 1991;34(1):127-139. (11 REFS)

A study was made of the applicability and specificity of three lymphocyte proliferation assays as biological markers for toxicant exposures. Tested compounds included cadmium chloride, potassium chromate, nickel chloride, mercuric chloride, benzoquinone, hydroquinone, styrene oxide and Aroclor-1254. Assays using Fischer-344 rat spleen lymphocytes included B-lymphocyte stimulation by lipopolysaccharide (LPS) and T-lymphocyte stimulation by concanavalin-A (ConA) or by antigen in mixed lymphocyte culture (MLC). F-344 rats were exposed in-vivo to potassium chromate or cadmium chloride in drinking water for 6 weeks, and capacities of splenic lymphocytes to serve as effectors or antigenic cells in MLC were determined in-vitro. Sprague-Dawley rats were used for allogeneic cells in MLC reactions. Mitogenic responses of T-cells and B-cells were reduced by in-vitro exposure to toxicants at levels of 10mg/ml or more. At the lowest concentration, all compounds significantly altered the response of at least one mitogen assay. Addition of exogenous chromium or cadmium to ConA and MLC assays using splenocytes from rats exposed to 100 or 200mg/ml of either metal altered the cell responses in-vitro. It was concluded that lymphocyte proliferation assays may be useful biomarkers for exposure to a wide variety of toxicants and may be modifiable for detection of exposure to specific chemicals.

MISCELLANEOUS

66

MacPhee DG. SUBSTITUTION OF ANIMALS IN TOXICOLOGY TESTING--A RUSH TO JUDGEMENT? [letter] Med J Aust 1992 Feb 3;156(3):223-4.

No abstract.

67

Guggenheim B. MEDICINE AND ANIMAL EXPERIMENTS--SOME THOUGHTS AND FACTS. Schweiz Monatsschr Zahnmed 1992;102(1):112-8,138-9.

No abstract.

68

Takeuchi H, Baba M, Shigeta S. AN APPLICATION OF TETRAZOLIUM (MTT) COLORIMETRIC ASSAY FOR THE SCREENING OF ANTI-HERPES SIMPLIX VIRUS COMPOUNDS. J Virol Methods 1991;33(1-2):61-71.

A rapid and sensitive procedure was developed for in vitro evaluation of anti-herpes simplex virus (HSV) agents. The procedure is based on spectrophotometric assessment for viability of virus- and mock-infected cells via in situ reduction of a tetrazolium dye MTT, which was previously used for the detection of anti-human immunodeficiency virus (HIV) agents. Monolayer cells such as human embryonic fibroblast, VERO, or HeLa cells were not suitable for this purpose. Among the non-adherent cell lines examined for susceptibility to HSV type 1 (HSV-1), a B-lymphoblastoid cell line NC-37 was found to be the most sensitive. After optimization, the method proved to be as sensitive as plaque reduction. The system simplifies the assay procedure and thus permits the evaluation of larger numbers of compounds for anti-HSV-1 activity.

MUTAGENICITY

69

Dearfield KL, Auletta AE, Cimino MC, Moore MM. CONSIDERATIONS IN THE U.S. ENVIRONMENTAL PROTECTION AGENCY'S TESTING APPROACH FOR MUTAGENICITY. Mutat Res 1991;258(3):259-284.

The paper provides the rationale and support for the decisions the Office of Pesticides and Toxic Substances of the Environmental Protection Agency (OPP) will make in requiring and reviewing mutagenicity information.

The regulatory requirement for mutagenicity testing to support a pesticide registration is found in the 40 CFR Part 158.

70

Claxton LD, Houk VS, Allison JC, Creason J. EVALUATING THE RELATIONSHIP OF METABOLIC ACTIVATION SYSTEM CONCENTRATIONS AND CHEMICAL DOSE CONCENTRATIONS FOR THE SALMONELLA SPIRAL AND PLATE ASSAYS. *Mutat Res* 1991; 253(2):127-136.

A factorial experimental design was used to evaluate the influence of multiple metabolic activation system concentrations on the dose-response exhibited by promutagens (indirect-acting mutagens) in the Salmonella spiral and plate assays. The mutagenic activity of the three compounds used spanned three orders of magnitude. The activity of the three promutagens tested ranged from 10 to 100 revertants/mug. During a single experiment a mutagen was tested in TA100 at 13 doses plus a negative control dose. Each dose was tested at 10 S9 concentrations. The spiral Salmonella assay, an automated version of the standard assay, generates dose-response data from a concentration gradient on a single agar plate. The study demonstrates not only that even small differences in S9 concentrations can affect the measurement of mutagenic potency but that S9/compound interactions cannot be generalized through the use of interaction studies. The study also demonstrates that spiral assay data and plate assay data for promutagens cannot be compared directly unless the S9 concentrations for all chemical doses are also comparable.

71

Claxton LD, Houk VS, Warner JR, Myers LE, Hughes TJ. ASSESSING THE USE OF KNOWN MUTAGENS TO CALIBRATE THE SALMONELLA TYPHIMURIUM MUTAGENICITY ASSAY: II. WITH EXOGENOUS ACTIVATION. *Mutat Res* 1991;253(2):149-160.

To determine the usefulness of selected chemicals as potential reference materials for calibrating the Salmonella assay, two laboratories tested a series of Salmonella mutagens that require exogenous activation. The purpose of this project was to evaluate the variability in the mutagenic response of potential reference chemicals that require exogenous metabolic activation in the standard plate-incorporation Salmonella mutagenicity assay, and to develop ranking criteria for mutagenic activity based on these data. Ten indirect-acting mutagens were tested in two

laboratories using *Salmonella typhimurium* TA100 and an Aroclor-induced rat liver S9. Each laboratory conducted four rounds of testing. A different batch of S9 was utilized for every two rounds. The use of ten closely spaced, nontoxic doses allowed for along with the data generated previously (Claxton et al., 1991b) for 10 direct-acting chemicals, provide a basis for intralaboratory and interlaboratory comparisons and rankings of *Salmonella typhimurium* TA100 mutagenicity data. Concurrent testing (same day, same laboratory, same technician, same bacteria, S9 and media) is a more acceptable method to control variance, but not always possible. The ability to quantify *Salmonella* mutagenicity data by the use of standard reference materials as those identified will assist in comparisons of potency data generated in different laboratories or within the same laboratory on different days.

72

Rosenkranz HS, Zhang YP, Klopman G. IMPLICATIONS OF NEWLY RECOGNIZED RELATIONSHIPS BETWEEN MUTAGENICITY, GENOTOXICITY AND CARCINOGENICITY OF MOLECULES. *Mutat Res* 1991;250(1/2):25-33. (43 REFS)

The CASE structure/activity relational system was used to simulate the properties of different populations of molecules with varying chemical prevalences through consideration of in-vitro and cancer bioassays and a *Salmonella* mutagenicity assay. The mutagenicity, cytogenotoxicity, carcinogenicity, sensory irritation, male rat specific alpha-2-mu nephrotoxicity, and maximum tolerated dose of a population of molecules were predicted. The chemicals (therapeutic agents, natural products, physiological chemicals, and other substances) were sorted by their predicted responses to specific tests. Predicted properties were evaluated and overlapping test results were studied. The authors concluded that the method facilitates determination of the dependence among assays and indicates levels of false positive and false negative predictions.

73

Ashby J, Tennant RW. DEFINITIVE RELATIONSHIPS AMONG CHEMICAL STRUCTURE, CARCINOGENICITY AND MUTAGENICITY FOR 301 CHEMICALS TESTED BY THE U.S. NTP. *Mutat Res* 1991;257(3):229-306.

Correlations were evaluated among chemical structure, mutagenicity to *Salmonella*, and carcinogenicity to rats and mice using 301 chemicals tested by the United States

National Toxicology Program. A high degree of correlation was noted between structural potential to DNA reactivity and mutagenicity. The correlation of either property with carcinogenicity was low. Correlations were then sought between different subgroups of compounds. Most of the rodent carcinogens were among the structurally alerting chemicals. Most of the structurally alerting chemicals were mutagenic. The authors conclude that structural alerts and mutagenicity to *Salmonella* are useful but nondefinitive indicators of the overt carcinogens in the database. Genotoxic carcinogens may be predicted by reference to chemical structure and the careful use of data generation by in-vitro and in-vivo genotoxicity assays. Nongenotoxic carcinogens require basic studies to understand the subtle effects that occur in rodents in protracted dosing with these chemicals.

74

Sarkar FH, Radcliff G, Callewaert DM, Marnett LJ, and others. MUTAGENIC RESPONSE OF MOUSE LYMPHOMA CELLS AFTER ACTIVATION OF BENZIDINE AND 2-AMINOFLUORENE WITH PURIFIED PROSTAGLANDIN H SYNTHAS. *Mutat Res* 1990;242(4):319-328.

A mouse lymphoma cell system using purified prostaglandin-H- synthase (PHS), rather than ram seminal vesicle microsomes, as the activating enzyme system was developed and tested on the aromatic amine carcinogens benzidine (BNZ) and 2-aminofluorene (2-AF). L5178Y-mouse-lymphoma TK3.7.2-C cells were used. BNZ by itself or in the presence of 5-phenyl-4-pentenyl-hydroperoxide (PPHP) induced a weak mutagenic response in mouse lymphoma cells. The addition of PHS or PHS and its cofactor PPHP increased the response five fold. A maximal mutagenic response for BNZ was noted after incubation with the complete activating system. When 2-AF was incubated with mouse lymphoma cells only a minimal mutagenic response was noted. Incubation of 2-AF with either PPHP or PPHP and PHS produced a significant enhancement of the mutagenic response. It was concluded that a purified mammalian peroxidase, PHS, can be used to activate aromatic amines in mouse lymphoma mammalian cell mutagenesis assays. The conditions provide standard protocols that can be used to screen a variety of aromatic amines for mutagenicity toward mammalian cells in conjunction with peroxidase activation.

NEUROTOXICITY

75

van der Maazen RWM, Verhagen I, van der Kogel AJ. AN IN VITRO CLONOGENIC ASSAY TO ASSESS RADIATION DAMAGE IN

RAT CNS GLIAL PROGENITOR CELLS. Internat J Rad Biol 1990; 58(5):835-844.

A clonogenic assay for evaluating radiation damage in rat central nervous system (CNS) glial progenitor cells was developed. Monolayers of type-1 astrocytes obtained from the cerebral cortex of 1 day old Wistar-rats were prepared by enzymatic digestion. After purification and irradiation with 20 gray (Gy) X-rays to prevent the growth of any remaining clonogenic O-2A cells, the monolayers were overlaid with suspensions of cells from the optic nerve, spinal cord, or any other part of interest from the Wistar-rat CNS. After being irradiated experimentally, the cultures were stained with appropriate monoclonal antibodies and a conjugate of goat antimouse immunoglobulin-M and fluorescein. After staining, the cells were fixed, washed, and dried. The extent of glial progenitor cell damage was determined from the amount of fluorescence as measured by a microscope with an epifluorescence attachment. The technique was applied to Wistar-rat optic nerve O-2A progenitor cells that had been irradiated in-vivo or in-vitro. In-vivo and in-vitro irradiated cells showed similar radiosensitivities. It was concluded that the assay can be used to examine the intrinsic radiosensitivity, repair, and regeneration of glial progenitor cells irradiated in-vitro or in-vivo.

76

Sawyer TW, Weiss MT, Boulet CA, Hansen AS. TOXICITY OF ORGANOPHOSPHATE NERVE AGENTS AND RELATED PHOSPHONYLATED OXIMES COMPARED TO THEIR ANTICHOLINESTERASE ACTIVITY IN NEURON CULTURES. Fund Appl Toxicol 1991;17(1):208-214.

A study was made of the toxicity and anticholinesterase activity of six organophosphorus nerve agents and sphonylated oxime derivatives, and in-vivo inhibition of acetylcholinesterase (AChE) was compared with in-vitro inhibition in primary neuron cultures. Tested compounds included VX, sarin, tabun, soman, diisopropyl-fluorophosphate and cyclohexyl-methylphosphonofluoridate and several oxime derivatives. Male and female CD-1-mice were injected subcutaneously with various doses of compounds and were observed for time to the appearance of symptoms of poisoning and to death. Median lethal doses (LD50s) were calculated, and blood was collected for AChE assay. Cortical neurons were obtained from embryos of pregnant CFW-mice and cultured for 12 to 15 days. Cultures were exposed to various doses of compounds for 15 minutes and AChE assayed for determination of median inhibitory concentrations (IC50s). Typical signs of classical organophosphate poisoning were observed and

were dose related as to severity. Time to signs of poisoning and death decreased with increasing doses. AChE levels in 24 hour survivors were comparable to control levels, while levels in dying mice were usually 2% or less than controls. Similar results were observed for in-vitro AChE inhibition in neuron cultures, and in-vitro IC50s were highly correlated with LD50s. It was concluded that mouse embryo neuron cultures may be a good model for studying organophosphates and potential antidotes.

77

Husain K. PERIPHERAL BIOCHEMICAL MARKER FOR ORGANOPHOSPHATE: INDUCED DELAYED NEUROTOXICITY. *Biochem Int* 1991;24(6):1051-1056.

Neurotoxic esterase (NTE) activity was assayed in platelets of human and mice, as well as in the brain of mice in vitro and in vivo. Mipaflox, a well known organophosphate toxicant was used to induce delayed neurotoxicity, at doses of 5, 10, and 15 mg/kg, subcutaneously, as a means of examining the relationship between inhibition of brain and platelet NTE activity in mice. It was observed that the platelet NTE activity of mice was less than in humans. The results indicate that mipaflox produces a dose dependent inhibition of brain and platelet NTE activity in vivo and concentration dependent inhibition in vitro. The assay of platelet NTE may prove a useful peripheral biochemical marker for organophosphate-induced delayed neurotoxicity.

78

Stevens CW. ALTERNATIVES TO THE USE OF MAMMALS FOR PAIN RESEARCH. *Life Sci* 1992;50(13):901-12. (72 REFS)

The study of pain and analgesia is an important area of biomedical research. This area of research examines the physiology of pain transmission and the pharmacology of analgesic drugs by employing a variety of in vitro and in vivo animal models. To date, the vast majority of in vivo models for pain research have used mammalian species, primarily rodents and, to a lesser extent, canines, felines, and primates. Existing literature on pain research using non-mammalian vertebrates is reviewed, with a special focus on amphibian species. Further development of non-mammalian models for pain research should be encouraged.

79

Smith RA. PRIMARY CULTURES OF ADULT MAMMALIAN SENSORY NEURONS AND OTHER IN VITRO SYSTEMS OF USE IN NEUROTOXICOLOGICAL STUDIES. Arch Toxicol (Suppl VOL 14, Recent Dev Toxicol: Trends, Methods Probl) 1991;14:8-14.

Several endpoints are chosen to observe toxic insult: cell viability, membrane integrity, neurite retention, ultrastructural changes, and protein leakage. Obvious caution is necessary in extrapolation from in vitro findings to possible in vivo effects. In vitro methods add understanding of the mechanisms of toxic action. They offer alternative approaches in the neurological investigations hence complementing in vivo studies.

OCULAR TOXICITY

80

Bruner LH, Kain DJ, Roberts DA, Parker RD. EVALUATION OF SEVEN IN VITRO ALTERNATIVES FOR OCULAR SAFETY TESTING. Fund Appl Toxicol 1991;17(1):136-149.

A statistical analysis compared seven in-vitro methods with in-vivo results of ocular safety assessment of 17 test materials. In-vitro assays included the silicon microphysiometer (SM), luminescent bacteria toxicity test (LBT), neutral red assay (NR), total protein assay (TP), Tetrahymena-thermophila motility assay (TTMA), bovine eye chorioallantoic membrane assay (BECAM) and the EYTEX system (ETS). The commercial EYTEX system was used in its standard form and as the membrane partition assay. Results were compared with known in-vivo low volume eye test (LVET) data for rabbits. Significant correlations between LVET and in-vitro assays were found for SM (r-value -0.87), LBT (r-value -0.91), NR protocols (r-values -0.89 and -0.85), TP (r-value -0.86) and TTMA (r-value 0.78). BECAM classified 15 substances as slight irritants and one each as moderate and severe. EYTEX showed no correlation with LVET data (r-value -0.29). It was concluded that five of the in-vitro assays may be useful for preliminary ocular safety screening to classify materials into broad irritancy categories.

81

Sugai S, Murata K, Kitagaki T, Tomita I. STUDIES ON EYE IRRITATION CAUSED BY CHEMICALS IN RABBITS: III. AN IN VITRO TESTING METHOD USING RAT RED BLOOD CELLS FOR THE PREDICTION OF EYE IRRITATION POTENTIAL OF CHEMICALS. J Toxicol Sci 1991;16(3):131-144.

Rat red blood cells were used as an in vitro method to evaluate the eye irritation potential of chemicals in rabbits. The results using 116 chemicals of various use categories were analyzed for the prediction of

possibility of eye irritation potentials. Eye irritation potential of chemicals was examined according to Draize method and chemicals were classified into three categories, (1) non or mild irritants, (2) moderate or severe irritants and (3) strong or corrosive irritants. The in vitro method consisted of two methods detecting the effects of chemicals mainly on protein and lipid in the membrane, which were evaluated by the induction of methemoglobin and hemolysis, respectively. The multivariate estimation by the above two in vitro data sets were 77.6% predictive of the in vivo classification.

82

Sugai S, Murata K, Kitagaki T, Tomita I. STUDIES ON EYE IRRITATION CAUSED BY CHEMICALS IN RABBITS: II. STRUCTURE-ACTIVITY RELATIONSHIPS AND IN VITRO APPROACH TO PRIMARY EYE IRRITATION OF SALICYLATES IN RABBITS. J Toxicol Sci 1991;16(3):111-130.

Structure-activity relationships and in vitro evaluation of eye irritation potential of salicylates in rabbits were studied. The primary eye irritation potential of ten salicylates was evaluated according to Draize method. The effects of chemicals on a model protein and on a lipid membrane were investigated in vitro. Effects on the protein could be detected by the production of aggregates of human serum gamma-globulin (HSG) and good correlation was obtained between the ability of salicylates to produce aggregation of HSG and the potential of corneal irritation. The effects on the lipid could be detected by the adhesion potential of chemicals on lipid membrane but no correlation was obtained between the adhesiory effects of salicylates on lipid membrane and potential for eye irritation. The corneal irritation and protein aggregation potential of salicylates were correlated with the acid dissociation constant more closely than with the octanol/water partition coefficient. Results suggest that eye irritation caused by salicylates are mainly the results of denaturation of proteins in ocular tissue and that the effects on protein depend on the dissociation potential of salicylate molecules.

83

Ali A, Sharma SN. FABRICATION OF A THROUGH-FLOW APPARATUS FOR IN-VITRO DETERMINATION OF DRUGS FROM OPHTHALMIC PREPARATIONS. Indian Drugs 1992;29(4):157-60.

An in vitro through-flow apparatus was developed as means for the in vitro evaluation of drugs in

ophthalmic preparations. Ocular inserts of Na cromoglycate were prepared by casting on mercury surface. In vivo release of drug from the ocular inserts was determined in rabbits' eye and release rate of drug was then determined at different flow rates in the apparatus. Results show high correlation between the in vitro and in vivo techniques for ocular inserts or other ophthalmic preparations. e.g., ointment, gels, liposomal eye products, etc.

ORGAN CULTURE

84

Ayoubi S, Ward P, Naik S, Sankaran M. THE USE OF PLACENTA IN A MICROVASCULAR EXERCISE. *Neurosurgery* 1992 Feb;30(2):252-4.

Twenty-five human placentas were studied for suitability in a microvascular surgical exercises. The size and useful length of vessels were measured, and different microvascular anastomoses were performed. The size of the placenta vessels compare with reported sizes of intracranial arteries. It was suggested that neurosurgeons use human placentas as complementary to experimental animals in microvascular training.

85

Reddy MV, Blackburn GR, Irwin SE, Kommineni C, et al. A METHOD FOR IN VITRO CULTURE OF RAT ZYMBAL GLAND: USE IN MECHANISTIC STUDIES OF BENZENE CARCINOGENESIS IN COMBINATION WITH 32P-POSTLABELING. *Environ Health Perspect* 1989;82:239-247.

An investigation was conducted using a nuclease-p-1 enhanced phosphorus-32 post labeling assay to evaluate the metabolism of the rat Zymbal gland in vitro. The ability of the gland to metabolize benzene and its derivatives, as well as 7,12-dimethylbenzanthracene (DMBA) and 2-acetylaminofluorene (AAF) was determined by measuring the covalent bonding of these compounds to DNA. Aromatic DNA adducts were detected in gland fragments exposed for 48 hours to benzene, its derivatives, DMBA, and AAF. The DNA adducts detected in the Zymbal gland indicates that this organ is capable of metabolically activating electrophilic intermediates that can bind to DNA. The authors concluded that the in vitro activation pathways in cultured Zymbal glands appear to be similar to those observed in results from in vivo investigations.

86

Brendel K, Fisher RL, Krumdieck CL, Gandolfi AJ.

PRECISION-CUT RAT LIVER SLICES IN DYNAMIC ORGAN CULTURE FOR STRUCTURE-TOXICITY STUDIES. J American College Toxicol 1990;9(6):621-627.

A review was presented which focused on the development of precision cut liver slice technology and the application of this method to the study of in vitro structure toxicity relationships. This in vitro mammalian liver system apparently simplified both metabolism and hepatotoxicity studies in several species. The basis of the method was the cutting of slices with high precision and the culturing of slices under dynamic organ culture conditions. It was noted that the utility of this method allows study of the intoxication and metabolism for several hours to days and can be used to help integrate biochemical assays and histological and morphological evaluations and structure toxicity studies.

REPRODUCTIVE TOXICITY

87

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-TESTOS: BARRIER IN VITRO. Toxicol Appl Pharmacol 1992;112(1):51-7.

The effect of various doses (0.75-24 microM) of cadmium chloride (CdCl₂) on the development of intercellular tight junctions by immature rat Sertoli cells (Sc) was investigated in vitro using a 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 CdCl₂ effects, the TER changes were correlated with Sc secretory activity (immunoactive inhibin), the cell number (DNA content), and viability (MTT test). The effects of CdCl₂ 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). The data suggest that CdCl₂ may selectively compromise, at least in vitro, the development and maintenance of the inter-Sc tight junctions, without affecting the secretory activity or the cell number and viability. However, increasing cumulative doses of CdCl₂ (concentration multiplied by the time of exposure) led to decreased inhibition of secretions and cell viability and then, finally, to irreversible cell damage and death. The experimental model and approach reported in this paper should prove 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.

88

Chiu TT, Tam PP. CONTAMINATION OF CULTURE MEDIUM WITH ENDOTOXIN AFFECTS EMBRYONIC DEVELOPMENT IN VITRO. Aust J Med Lab Sci 1991;12(4):130-133.

A study was carried out to relate the incidence of endotoxin-contaminated culture medium to the poor outcome of in vitro fertilization. Two sources of endotoxin were identified: the serum samples and the water used for preparing the culture medium. Retarded embryonic development and cytoplasmic fragmentation were associated with endotoxin-contaminated medium. Six on-going pregnancies were obtained from in vitro fertilization during the period of investigation, none of which involved endotoxin-contaminated cultures.

89

Holloway AJ, Moore HDM, Foster PMD. THE USE OF RAT IN VITRO FERTILIZATION TO DETECT REDUCTIONS IN THE FERTILITY OF SPERMATOOZOA FROM MALES EXPOSED TO ETHYLENE GLYCOL MONOMETHYL ETHER. Reprod Toxicol 1990;4(1):21-27. (19 REFS)

The ability of an in-vitro fertilization assay to detect changes in the fertility of spermatozoa after exposure to ethylene-glycol-monomethyl-ether (EGME), a testicular toxicant, was evaluated using male Wistar-rats. Single, oral doses of EGME were administered to male rats at 50 to 200mg/kg in order to induce lesions in germinal epithelium. Spermatozoa were retrieved from the cauda epididymides of these males at fixed intervals following treatment, and used subsequently to inseminate oocytes from immature female Sprague-Dawley rats. Oocytes and embryos were then examined by phase contrast microscopy for any evidence of infertility. The fertility of control males was found to be consistently above 65% in the presence of a spermatozoa concentration of at least 300,000 per milliliter. In contrast, spermatozoa from males treated with EGME exhibited a dose dependent reduction in fertilizing capacity at specific times after the drug administration. Fertility reductions between 2 and 3.5 weeks, 4.5 to 6 weeks and at 7 weeks post administration corresponded to damage to elongated spermatids, pachytene spermatocytes and eptotene (also preleptotene) spermatocytes, respectively. The authors conclude that this in-vitro fertilization assay can detect spermatozoa infertility attributable to alterations in the function, as well as the number of sperm. Since there are an increasing number of reports of reduced fertility in humans from environmental or occupational exposure and that the in-vitro

fertilization assay may detect fertility changes at lower doses of toxicant than conventional serial breeding, it is thought that this approach warrants further evaluation.

90

Ernst E, Christensen M, Lauritsen J-G. IN-VITRO EXPOSURE OF HUMAN SPERMATOZOA TO MERCURIC CHLORIDE-A HISTOCHEMICAL STUDY. Graumann, W. (ED.). Progress in Histochemistry and Cytochemistry, 23(1-4),1990.

No abstract.

91

Hughes SF, Haney AF, Hughes CL Jr. USE OF HUMAN CUMULUS GRANULOSA CELLS FOR IN VITRO SCREENING OF REPRODUCTIVE TOXICANTS. Reprod Toxicol 1990;4(1):11-15. (7 REFS)

An investigation was made into the possibility of using human cumulus granulosa cells to screen for putative reproductive toxins in-vitro. Human granulosa cells were obtained from the cumulus cell mass during the process of human in-vitro fertilization and embryo transfer. Progesterone levels, critical for the establishment and maintenance of pregnancy, were measured by radioimmunoassay, and used to assess granulosa cell function. Supplementation of the media with human chorionic gonadotropin (hCG) was found to be necessary in order to preserve constant progesterone production in-vitro. A number of estrogenic agents, including 17-beta-estradiol, clomiphene-citrate and o,p-DDT, were shown to significantly impair the hCG-assisted progesterone production in this in-vitro system. Although a comprehensive characterization of these cells is required for the validation of this technique, it was concluded that the results do support the use potential of granulosa cells from human cumulus for the in-vitro screening of possible female reproductive toxicants.

92

Kamiguchi Y, Tateno H, Mikamo K. MICRONUCLEUS TEST IN 2-CELL EMBRYOS AS A SIMPLY ASSAY SYSTEM FOR HUMAN SPERM CHROMOSOME ABERRATIONS. Mutat Res 1991;252(3):297-303.

An attempt was made to incorporate the micronucleus test into the human sperm/hamster oocyte fertilization system for assessing radiation induced chromosomal damage in human spermatozoa. A comparison was made of the incidence of micronuclei in two cell embryos with the incidence of structural chromosome aberrations in human spermatozoa to ascertain the efficiency of the micronucleus test. Semen

samples were obtained from a healthy man and subsequently exposed in-vitro to 1.11 and 2.13 grays (Gy) of cesium-137 gamma rays at a dose rate of 1.36Gy/minute. Total numbers of 155 and 142 monospermic eggs were obtained in the 1.11Gy and 2.13Gy groups, respectively. Among these, 95 eggs and 98 eggs, respectively, developed successfully to the two cell stage. A positive correlation was noted between the size of the micronucleus and the number of nucleoli. Total numbers of 159 and 145 sperm derived chromosome complements were karyotyped in the low and high dose groups, respectively. The number of spermatozoa with structural chromosome aberrations was 84 (52.8%) and 115 (79.3%) in the two groups, respectively. Types of structural chromosome aberrations observed were breaks, fragments, gaps and exchanges. A correlation was noted between the number of micronuclei per embryo and the number of breaks and fragments per spermatozoon. It was concluded that the method is useful for assessing chromosomal damage in human spermatozoa.

SKIN

93

Pape WJ, Hoppe U. IN VITRO METHODS FOR THE ASSESSMENT OF PRIMARY LOCAL EFFECTS OF TOPICALLY APPLIED PREPARATIONS. *Skin Pharmacol* 1991;4(3):205-12.

Three 'alternative' methods for the screening of the primary irritation potentials of topically applied preparations and raw materials are presented as a test battery and their in vitro endpoints are compared with in vivo data. The first of these defined methods is the intermediate test on the chorioallantoic membrane of hens' fertilized eggs and which is generally proposed for the prediction of the irritation potential of chemicals. The second, involves cytotoxicity tests with Balb/c 3T3 fibroblasts that are used to characterize the influence of

test materials on cellular homeostasis and viability. The third means of in vitro evaluation is a rapid photometric red blood cell assay, which permits distinguishing between damage to the membrane and to proteins as endpoints which correlate with lesions observed on the conjunctiva, iris and cornea in the eye and the acute inflammatory responses evoked during epicutaneous irritancy testing. These methods are proposed and can be used advantageously for testing newly developed topical preparations and their ingredients in view of their local irritation potential without further use of animal testing. They can also be used for screening chemicals.

94

Kemppainen BW, Reifenrath WG, Stafford RG, Mehta M.
METHODS FOR IN VITRO SKIN ABSORPTION STUDIES OF A
LIPOPHILIC TOXIN PRODUCED BY RED TIDE. *Toxicology*
1991;66(1):1-17.

The penetration and distribution of the red tide brevetoxin PbTx-3 in pig skin was studied in-vivo and in-vitro. Weanling female Yorkshire-pigs were administered tritium (H-3) labeled PbTx-3 topically or subcutaneously (sc) as a preliminary range finding experiment. Approximately 36 and 80% of the doses were recovered after sc and topical administration, respectively. After sc administration approximately 20% of the dose was eliminated in the urine and feces. After topical application 2.3% of the dose was eliminated in the urine and feces. Approximately 34% of the dose was found on the skin surface and 39% was removed along with the protective patches. In-vivo, accumulation of PbTx-3 in the epidermis was greater than in the dermis at all time points. After correcting for incomplete excretion of radiolabel, the total skin penetration amounted to 11.5% of the dose. Skin sections removed from untreated pigs were mounted on diffusion cells and treated with radiolabeled PbTx-3 for 48 hours. In-vitro, the amount of H-3 accumulated in the dermis was significantly greater than in the epidermis at all times except at 48 hours. When the amount of radioactivity remaining in the dermis was also considered the mean cutaneous absorption of PbTx-3 was 9.9%. It was concluded that measurements of the in-vitro skin penetration of PbTx-3 significantly underestimate in-vivo penetration when they are based only on radioactivity accumulating in the receptor fluid.

TERATOGENICITY

95

Aulthouse AL, Carubelli CM, Dow TM, Ziegelmayr C, Beck M. INFLUENCE OF RETINOL ON HUMAN CHONDROCYTES IN AGAROSE CULTURE. *Anat Rec* 1992;232(1):52-59.

Vitamin A and its congeners, collectively called retinoids, are known to have teratogenic potential and to have induced craniofacial and limb malformations in unnumerous animal species. More importantly, retinoids are recognized as teratogenic to fetuses of pregnant women who have taken such preparations for dermatologic disorders. Information gathered from the study of animal models suggests that retinoids interfere with cartilage differentiation. In vitro studies using various animal systems have shown that cartilage matrix macromolecules are altered to resemble those secreted by mesenchymal cells. The response of human chondrocytes to retinoids in vitro is not known.

Culture of human chondrocytes in agarose maintains the cartilage phenotype and therefore serves as a model system. The studies presented in this paper were done to determine if the expression of specific matrix macromolecules of human chondrocytes in agarose culture is altered by retinol treatment. Results indicate that the chondrocytes are altered by retinol in vitro.

96

Takashima H, Kuwagata N, Wada A, Nagao T, Mizutani M.
AN APPROACH TO IN-VITRO TERATOGENESIS SCREENING USING WHOLE EMBRYO CULTURE I. EFFECT OF BASE ANALOGS ON RAT EMBRYOS. 31ST ANNUAL MEETING OF THE JAPANESE TERATOLOGY SOCIETY, IZUMO, JAPAN, JULY 11-12, 1991. Congenital Anom 1991;31(3):259.

No abstract.

TOXICITY (General)

97

Ahne W, Halder M. R1 FISH CELL CULTURE FOR DETERMINING WASTEWATER TOXICITY IN MUNICH CONTRIBUTIONS TO WASTEWATER, FISHERY AND RIVER BIOLOGY, VOL. 45. CURRENT CHEMICAL AND BIOLOGICAL WATER AND SLUDGE ANALYSIS: APPLICATION, RESULTS AND THEIR ECOLOGICAL EVALUATION. 411P. R. OLDENBOURG VERLAG: MUNICH, GERMANY, 1991.

No abstract.

98

Frazier JM. ORGANIZATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT (OECD) SCIENTIFIC CRITERIA FOR VALIDATION OF IN VITRO TOXICITY TESTS. The Johns Hopkins University, School of Hygiene and Public Health, 615 N. Wolfe Street, Baltimore, MD 21205, USA, 1991.

This document was prepared for the Organization for Economic Co-operation and Development (OECD) following their request for a general document on the validation of in vitro methods. Contents: introduction (uses and needs for toxicity testing, safety evaluation and toxicity testing, comparison of in vivo and in vitro toxicity testing); overview of validation processes; selection of tests for validation; selection and reference classification of chemicals for validation; technical problems associated with validation studies, intralaboratory assessment and test database development; criteria for test evaluation; battery selection; in vitro toxicity testing and human safety assessment; case study of ocular irritation testing.

99

Liu D, Kwasniewska K, Chau YK, Dutka BJ. A FOUR-HOUR AGAR PLATE METHOD FOR RAPID TOXICITY ASSESSMENT OF WATER-SOLUBLE AND WATER-INSOLUBLE CHEMICALS. *Environ Toxicol Water Qual* 1991;6(4):437-444.

No abstract.

VALIDATION

100

Grilli S, Bartoli S, Bonora B, Colacci A, et al. GENOTOXICITY OF CHLOROETHANES AND STRUCTURE-ACTIVITY RELATIONSHIPS. *Chem Carcinog* 2 [Proc. 5th Sardinian Int. Meet. Modulating Factors Multistage Chem Carcinog], 1991;381-91.

The aim of this study was to better identify the genotoxic activity of chloroethanes in in vivo and in vitro systems, in an attempt to validate a cell-free system as a short-term test for carcinogenicity prediction of initiating compounds, and to find structure-activity relationships among compounds belonging to the same chemical classification.

101

Riley RT. MYCOTOXICOSES, IN VITRO AND IN VIVO: CONJECTURE-HYPOTHESIS-VALIDATION. *Biodeterior Res* 3 [Proc 3rd Meet. Pan Am Biodeterior Soc], 1990;11-20.

In the field of mycotoxicology, it is difficult to maintain focus on the mycotoxin contamination of food and feed safety because the chemical and biological diversity of the subject matter reveals an infinite number of exciting and rewarding areas for basic research, while the epidemiological database that supports the hypothesis that fungal toxins in the blood supply are a threat to American consumers is extremely limited or nonexistent. The in vitro data can not stand by itself to demonstrate risk but can lead to implications of safety methods needed. The animal risks are more apparent. Accumulation of fungal metabolites results in acute toxicity or carcinogenicity. All researchers who work with fungal metabolites frequently speculate on the potential of fungal metabolites in the food and feed to alter immunity, reproduction, development, behavior, kidney and liver function. For basic researchers, the goal of communicating the concept of risk to the public is most likely unrealistic. However, within the realm of possibility, is the capability of other scientists to communicate

the concepts of risk based on in vitro and in vivo studies.