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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.

CANCER

1

Dahlberg WK, Little JB, Fletcher JA, Suit HD, Okunieff P. RADIOSENSITIVITY IN VITRO OF HUMAN SOFT TISSUE SARCOMA CELL LINES AND SKIN FIBROBLASTS DERIVED FROM THE SAME PATIENTS. Int J Radiat Biol 1993; 63(2):191-8.

Skin fibroblast cell strains and tumour cell lines were established from 12 patients with various types of soft tissue neoplasms, and radiation survival curve parameters were measured in vitro. Soft tissue sarcoma cells were consistently more sensitive to X-irradiation than fibroblasts isolated from the same patient, and were also more sensitive as a group than cell lines derived from 34 other human tumours. There was a general correlation in radiosensitivity between fibroblasts and tumour cells derived from the same patient, indicating that some component of tumour cell sensitivity may relate to genetic factors in the host. Such genetic factors, however, do not explain all of the heterogeneity in tumour cell response. The response of soft tissue sarcoma in vivo may be dependent on complex radiomodifying factors other than inherent radiation sensitivity, thus making it difficult to predict clinical outcome by use of assays which use survival of irradiated tumour cell lines in vitro as an endpoint.

2

Tashiro T. CELL CULTURE AND ITS APPLICATION. IN VITRO EVALUATION OF ANTICANCER ACTIVITY USING HUMAN TUMOR CELL LINES. Gan to Kagaku Ryoho 1992;19(12):2107-12.

Selective toxicity against cancer cells is an important determinant for anticancer agents. The authors evaluated anticancer effects in vivo using murine tumor models for several decades. Approximately 50 anticancer agents are currently available for clinical therapy, but very few agents are effective against some types of cancer. Much progress in cell culture techniques resulted in establishment of various human tumor cell lines. Currently, we are able to use human tumor lines as well as murine ones for the examination of drug sensitivity. A number of assay methods to evaluate anticancer activity have been developed. In the beginning, growth inhibitory activity was evaluated by counting cell numbers after drug exposure. Then, the human tumor clonogenic assay (HTCA) was designed to

measure only proliferative cells. Recently the colorimetric MTT assay and the SRB assay in 96-well microplates were developed, which were adopted in the screening system at the NCI, based on a new idea, i.e., disease-oriented screening (DOS) using about 60 human tumor cell lines. In this paper an outline of each method is described, especially with reference to disease-oriented screening.

3
Renier A, Yegles M, Buard A, Dong H, Kheuang L, Saint-Etienne L, Laurent P, Jaurand MC. USE OF MESOTHELIAL CELL CULTURES TO ASSESS THE CARCINOGENIC POTENCY OF MINERAL OR MAN MADE FIBERS. Cell Biol Toxicol 1992;8(3):133-9. (36 REFS)

Natural mineral fibers may produce pulmonary cancers and mesothelioma. In contrast with lung cancer, the incidence of fiber-induced mesothelioma is not enhanced in smokers compared to non smokers. It is therefore of special interest to use mesothelial cells to study the toxicity of natural or man made mineral fibers. Several years ago, the authors have developed a method to culture rat pleural mesothelial cells (RPMC). They first studied the effects of asbestos fibers by the application of in vitro tests formerly developed to determine the genotoxicity and transforming potency of soluble xenobiotics. The paper reviews the results obtained so far. It has been found that asbestos fibers produce a cell transformation and a genotoxicity characterized by the formation of aneuploid cells, abnormal anaphases, chromosomal aberrations and DNA repair effects. Experiments are now in progress to determine whether the in vitro effects observed are dependent on the fiber parameters suggested as playing a role in carcinogenic potency.

CARCINOGENICITY
4
Heil J, Reifferscheid G. DETECTION OF MAMMALIAN CARCINOGENS WITH AN IMMUNOLOGICAL DNA SYNTHESIS-INHIBITION TEST. Carcinogenesis (London) 1992;13(12):2389-94.

The Salmonella gene mutation assay (Ames test) is the most widely used test for the screening of mutagens. However, many in vitro tests hold unsatisfactory validity data, presumably because of the inability of present short-term tests to detect nongenotoxic

carcinogens, which are increasingly being brought into focus in the discussions of genesis of cancer. One principle often neglected in this context is the property of genotoxic agents to inhibit replicative DNA synthesis in (proliferating) eukaryotic cells. The authors believe that this early response to DNA damage is important in the multistage process of carcinogenesis. Accordingly, the authors proposed that

a DNA synthesis-inhibition test should be included in the test batteries for carcinogen screening. The development of an appropriate DNA synthesis-inhibition test based on immunological techniques is reported.

CELL CULTURE

5

Torishima H, Yamamoto R, Nishino T. IN VITRO TOXICITY TESTING USING ANIMAL CELLS. Seitai Zairyo 1992; 10(2):74-80.

A review with 49 refs. on the use of animal cell cultures as in vitro cytotoxicity test systems for chems. in pharmaceuticals, cosmetics, and other products. The use of human epidermal keratinocyte and rabbit corneal epithelial cell culture systems in the evolution of cytotoxicity of surfactants and preservatives is discussed.

6

Hoffman RM. THREE-DIMENSIONAL GEL-SUPPORTED NATIVE-STATE HISTOCULTURE FOR EVALUATION OF TUMOR-SPECIFIC PHARMACOLOGICAL ACTIVITY: PRINCIPLES, PRACTICE AND POSSIBILITIES. J Cell Pharmacol 1991; 2(4):189-201. (55 REFS)

A 3-dimensional sponge-matrix supported histoculture system is described that allows tissues to be cultured with the preservation of native tissue architecture and function. Over 25 types of human tumor types have been successfully cultured in this system with the maintenance of proliferative capacities, tumor architecture, and functions including tumor-stromal interactions. Normal tissues can also be cultured in the system with the maintenance of tissue architecture and proliferative capacity. A drug-response assay has been developed with this system that allows over 80% of specimens from all tumor types to be evaluated. Accurate in vitro/in vivo drug-response correlations have been obtained using the native-state system. The system offers low-cost

predictive drug-response determinations for cancer patients undergoing surgery or biopsy, as well as assays of other clinically-related parameters. The system ability to culture both tumor as well as normal tissue and to assess their drug-response spectra allows evaluations of tumor specificity of new anticancer agents.

7

Harauchi T, Hirata M. EFFECTS OF P-PHENYLENEDIAMINES AND ADRIAMYCIN ON PRIMARY CULTURE OF RAT SKELETAL MUSCLE CELLS. Toxicol Lett 1993;66(1):35-46.

In vitro toxicity of p-phenylenediamine derivatives and adriamycin were examined using cultured rat myofibers prepared by the selective plating method, with slight modification. When the myofibers were cultured for 2 days with 100 microMolar of N,N,N',N'-tetramethyl p-phenylenediamine, atrophy and/or swelling of the cells were observed, and the toxic effect was reduced as the number of N-methyl groups in the phenylenediamine molecule was decreased. Adriamycin, at concentrations of greater than 0.25 microM, caused cell injury. Decline of cellular creatine phosphokinase activities generally preceded the apparent morphological changes. The primary culture of rat myofibers responded sensitively to the agents which were myotoxic in vivo.

8

Brown DG, Willington MA, Findlay I, Muggleton-Harris AL. CRITERIA THAT OPTIMIZE THE POTENTIAL OF MURINE EMBRYONIC STEM CELLS FOR IN VITRO AND IN VIVO DEVELOPMENTAL STUDIES. In Vitro Cell Dev Biol 1992; 28A(11-12):773-8.

Cultured mouse embryonic stem (ES) cells are used for both in vitro and in vivo studies. The uncommitted pluripotent cells provide a model system with which to study cellular differentiation and development; they can also be used as vectors to carry specific mutations into the mouse genome by homologous recombination. The prolonged in vitro culture of rapidly dividing ES cells can lead to accumulated changes and chromosomal abnormalities that will compromise the biological function and abrogate germ line transmission of chimeric mice carrying novel genetic mutations. Such in vitro conditions will vary between individual

laboratories; for example, differences in the serums

used for maintenance. Using a number of different criteria the authors attempt in this paper to define the parameters that were found to be key factors for optimization of the biological potential of established ES cell lines. The successful integration into the germ line is dependant on acquiring or deriving a competent totipotent mouse ES diploid cell line. In this paper parameters and criteria are defined which were found to be key factors for the optimization of the biological potential of established ES cell lines.

9
Spier RE. CELL CULTURE SYSTEMS AND IN-VITRO TOXICITY TESTING TECHNICAL REPORT NO. 4 OF THE JOHNS HOPKINS CENTER FOR ALTERNATIVES TO ANIMAL TESTING. CAAT TECHNICAL WORKSHOP OF JUNE 13-15 1990. Cytotechnology 1992;8(2):129-136.

No abstract.

10
Zarutskie PW, Dixon LL, Hiller SL. IDENTIFYING SOURCES OF BACTERIAL ENDOTOXIN CONTAMINATION IN AN IN-VITRO FERTILIZATION IVF CULTURE ENVIRONMENT. J Assist Reprod Genet 1992;9(1):77-80.

No abstract.

11
Carrara M, Cima L, Cerini R, Dalle Carbonare M. AN IN VITRO METHOD FOR ASSESSING POTENTIAL TOXICITY OF COSMETIC PRODUCTS. J Toxicol, Cutaneous Ocul Toxicol 1993;12(1):3-13.

One difficulty encountered in testing finished cosmetic products arises from their final physical form. The presence of lipid or solid phases in the products makes them incompatible with the currently used cell culture media. In this study, a new method was used that permits the use of the testing materials directly on the cell (fibroblast) monolayer using a cell culture insert (Falcon). This device is characterized by a polycarbonate porous membrane at the bottom of the insert. The insert containing the testing samples is put on top of the well and is then in contact with the medium. In this way, it is still possible, after the exposure, to evaluate several parameters that are

indexes of cell injury. The toxicity induced by four cosmetic emulsion bases was tested using neutral red uptake detn., protein quantification, and lactate dehydrogenase activity on L929 cells. The least toxic emulsion was then used to estimate the toxicity of two commonly used preservatives, Kathon CG and Bronopol, at four different concentrations. Kathon CG showed a good dose-response relationship, while Bronopol inhibits neutral red uptake at all the tested concentrations. The protein determination performed on the same cells showed no significant difference between the two preservatives used, even when morphological changes were evident and the neutral red uptake was almost absent.

CENTRAL NERVOUS SYSTEM

12

Kuromi H. CHARACTERIZATION OF SUBSTANCES WHICH PROMOTE OR REPEL SYMPATHETIC FIBER GROWTH IN VITRO. Neurosci Res (Shannon, Irel) 1992;14(3):213-25.

To determine whether a recognition mechanism is involved in determination of sympathetic innervation patterns of various tissues, tissue-derived substances were applied to a restricted test surface region of dishes and the responses of cultured sympathetic neurites were examined. Sympathetic fibers exhibited a turning or ramifying response, resulting in a dense fiber growth on test regions coated with particulate (adheron) fractions of a conditioned-medium (CM) from expansor secundariorum, heart, peripheral blood vessel, or abdominal aorta; however, on test regions coated with adherons from lung, skeletal muscle, or dorsal aorta, the neurite growth was repelled and sparse fiber growth was observed. Experimentation results of suggest that adheron particles may participate in determination of sympathetic innervation patterns. Activity which repels or promotes the sympathetic fiber growth was inactivated by pronase E or trypsin but not by DNase or neuroaminidase. Repelling activity was lost after treatment with heparinase or heparitinase but not with chondroitinase ABC or hyaluronidase. Promoting activity was retained after treatment with these glycosidases. The results of these experiments suggest that the factor(s) possessing a repellent effect is a heparan sulfate proteoglycan and the factor possessing a promoting effect is a protein.

CLASTOGENICITY

13
Tadaki S, Nozaka T, Yamada S, Ishino M, Morimoto I, Tanaka A, Kunitomo J. CLASTOGENICITY OF APORPHINE ALKALOIDS IN VITRO. J Pharmacobio-Dyn 1992; 15(9):501-12.

The chromosomal aberration test using a Chinese hamster lung cell line (CHL) was carried out on 19 aporphine alkaloids including apomorphine with and without rat liver homogenates (S9) mix. Eighteen of 19 alkaloids tested induced chromosomal aberration in the presence or absence of S9 mix.

14
Matsuoka A, Yamazaki N, Suzuki T, Hayashi M, Sofuni T. EVALUATION OF THE MICRONUCLEUS TEST USING A CHINESE HAMSTER CELL LINE AS AN ALTERNATIVE TO THE CONVENTIONAL IN VITRO CHROMOSOMAL ABERRATION TEST. Mutat Res 1992; 272(3):223-36.

The in vitro micronucleus (MN) test was carried out simultaneously with the conventional chromosomal aberration (CA) test on 11 clastogenic chemicals or spindle poisons with different modes of action using a Chinese hamster cell line (CHL). The method of slide preparation for the MN test was the same as that for the conventional metaphase analysis, except that 1% acetic acid in methanol was used as the cell suspension medium for air-drying (to preserve the cytoplasm around the nucleus). All chemicals tested induced micronuclei reproducibly and were dose-dependently in good agreement with the results of metaphase analysis (r = 0.99). Since the MN test methodology is simple and the observation of MN is less subjective than that of CA, we conclude that the in vitro MN test would be a good alternative to the conventional CA test for screening the genotoxicity of chemicals.

15
Ciaravino V, Suto MJ, Theiss JC. HIGH CAPACITY IN VITRO MICRONUCLEUS ASSAY FOR ASSESSMENT OF CHROMOSOME DAMAGE: RESULTS WITH QUINOLONE/NAPHTHYRIDONE ANTIBACTERIALS. Mutat Res 1993;298(94):227-36.

A high capacity in vitro micronucleus assay was developed to evaluate the ability of selected 6-fluorinated quinolone and naphthyridone antibacterial compounds to induce micronuclei (MN) in vitro in V79 Chinese hamster lung cells.

CYTOTOXICITY

16
Balls M, Clothier RH. CYTOTOXICITY ASSAYS FOR INTRINSIC TOXICITY AND IRRITANCY. In Vitro Methods Toxicol 1992;37-52.

A review and discussion with 44 refs. on the development of cytotoxicity tests and their modification for special purposes and for testing certain types of materials. Three particular examples of general cytotoxicity tests (the FRAME kenacid blue test, the FRAME neutral red release test, and the FRAME fluorescein leakage test) are described. The current status and the future potential of the general cytotoxicity approach in in vitro toxicol. are also discussed.

17
Brandao JC, Bohets H HL, Van De Vyver IE, Dierickx PJ. CORRELATION BETWEEN THE IN VITRO CYTOTOXICITY TO CULTURED FATHEAD MINNOW FISH CELLS AND FISH LETHALITY

DATA FOR 50 CHEMICALS. Chemosphere 1992;25(4):553-562.

Neutral red uptake inhibition was investigated as an alternative method for the assessment of ecotoxicity. The 50 chemicals tested include different kinds of inorganic compounds and organic compounds such as alcohols, acids, ketones, esters, and others. The xenobiotics were applied at different concentrations to cultured FHM (fathead minnow fish) cells. After 2 hours contact time neutral red uptake inhibition was measured. The results are expressed as the NI50 value, which is the concentration of test compound required to induce a 50% reduction in neutral red uptake. The NI50 values were compared with fish lethality data (LC50) obtained in golden orfe by Juhnke and Ludemann (1978). For 49 of the chemicals a correlation coefficient r=0.89 was found. Hence it appears that the neutral red uptake inhibition in cultured fish cells can be considered as a valuable tool for in vitro ecotoxicity testing of a wide variety of chemicals.

18
Arditi M, Zhou J, Kim KS. IN-VITRO CYTOTOXICITY OF HAEMOPHILUS-INFLUENZAE TYPE B HIB AND HIB LIPOOLIGOSACCHARIDE (LOS) FOR BOVINE BRAIN ENDOTHELIAL

CELLS (BBEC). 32nd Interscience Conference on Antimicrobial Agents and Chemotherapy, Anaheim, California, USA, October 11-14, 1992. Program Abstr Intersci Conf Antimicrob Agents Chemother 1992; 32(0):268.

No abstract.

19
Kato I, Harihara A, Mizushima Y. AN IN VITRO MODEL FOR ASSESSING MUSCLE IRRITATION OF ANTIBIOTICS USING RAT PRIMARY CULTURED SKELETAL MUSCLE FIBERS. Toxicol Appl Pharmacol 1992;117(2):194-9.

The authors examined the possibility of using rat primary cultured skeletal muscle fiber to estimate the muscle irritation of antibiotics. The cells were exposed to cefaloridine (CER), cefazolin sodium (CEZ), flomoxef sodium (FMOX), cefamandole sodium (CMD), latamoxef sodium (LMOX), or cefalotin sodium (CET) at concentrations of 0 (control), 31.25, 62.5, 125, and 250 mg/ml in culture medium for 1 hr on Day 11 of culture. Cellular creatine kinase (CK) activity was measured as an indication of cell injury. The concentration of the antibiotic, at which CK activity decreased to 50% of the control (depletion concentration 50%, DC50), was utilized as an index of

cytotoxicity. DC50s of CER, CEZ, FMOX, CMD, LMOX, and CET were estimated to be 406.7, 311.1, 211.6, 132.7, 114.2, and 56.5 mg/ml, respectively. There was a good correlation between DC50 obtained in the in vitro test and the irritation volume in the in vivo test. The results suggest that the in vitro system using rat primary cultured skeletal muscle fibers is a useful alternative model for in vivo rabbit study to evaluate muscle irritation.

20
Chirila TV, Thompson DE, Constable IJ. IN VITRO CYTOTOXICITY OF MELANIZED POLY(2-HYDROXYETHYL METHACRYLATE) HYDROGELS, A NOVEL CLASS OF OCULAR BIOMATERIALS. J Biomater Sci Polym Ed 1992; 3(6):481-98.

Due to their ability to absorb ultraviolet and visible radiation, we have proposed the melanized poly(2-hydroxyethyl methacrylate) hydrogels as

biomaterials suitable for the manufacture of soft artificial intraocular lenses. Their biocompatibility has not been evaluated so far. In this study, poly(2-hydroxyethyl methacrylate) containing various amounts of adrenochrome-melanin were synthesized and the cytotoxicity of their aqueous extracts was assessed by using four in vitro testing techniques (trypan blue dye exclusion, inhibition of DNA synthesis, lactate dehydrogenase release, and inhibition of cell growth). Assays were based on incubation with human choroidal fibroblasts. The results suggest that the release of potentially toxic agents from melanized hydrogels into an aqueous medium is not significant. However, when an assay in collagen gel was carried out in the presence of specimens of melanized hydrogels, a toxic reaction was clearly revealed. This can be caused by a delayed release of toxic molecules from melanin, or by some other mechanism. The use of melanin-containing polymers as implant materials becomes questionable and further research is necessary.

21
Ferro M, Bassi AM, Penco S, Piana S, Usiglio D, Nanni G. COMPARATIVE ASSESSMENT OF THE CYTOTOXIC EFFECTS OF DIFFERENT XENOBIOTICS IN THREE HEPATOMA CELL LINES. Arzneim-Forsch 1992;42(8):1053-1057.

MH1C1, HTC and HEPA 1c1c7 hepatoma cells lines were selected in this study as the bioindicators of the cytotoxicity induced by six chemicals: butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), cycloheximide (CHE), cyclophosphamide (CPA), potassium dichromate (CrVI) and 2,4-dinitrophenol (DNP). The concentrations used were in the range from 10-6 mol/l to 10-2 mol/l, and the exposure time as 24 h. Two end-points were measured to evaluate cytotoxicity: the detachment of dead cells from the monolayer (CS), as evaluated by detection of the total macromolecules present in the cell monolayers solubilized in alkali and the loss of colony-forming efficiency (CF). The dose-response curves were different from one compound to another, but generally similar with the two assays, the colony formation being the most sensitive test. The sensitivity of the three cell lines was very similar, with some differences in the case of compounds exerting intermediate toxic effects, like CHE and DNP. The most toxic compound was Cr(VI), the least toxic one was CPA. The low cytotoxic effects displayed by CPA could be due to a lack of bioactivation and/or an increase of the inactivating enzymes, which are typical of hepatoma

cells lines.

22
Cruz AS, Figueiredo CA, Martinez C HO, Gomes L F DS.
MRC-5, HELA AND RC-IAL CELL LINES SENSITIVITY FOR DETECTION OF CYTOTOXICITY OF BIOCOMPATIBLE MATERIALS.
Rev Inst Med Trop Sao Paulo 1992;34(2):99-105.

The sensitivity of diploid and heteroploid cell lines for detection of cytotoxicity using the agar diffusion method on cell culture, was tested with ascorbic acid solution of different concentrations. A total of 562 samples of 21 various materials were tested.

23
Ekwall B, Nordensten C, Albanus L. TOXICITY OF 29 PLASTICIZERS TO HELA CELLS IN THE MIT-24 SYSTEM.
Toxicology 1982;24(3/4):199-210. (23 REFS)

The toxicity of plasticizers to HeLa cells was studied in-vitro. Twenty nine plasticizers representative of the types used in Swedish industry were incubated with HeLa cells for 7 days. Cytotoxicity was assessed using the Metabolic Inhibition Test-24 (MIT24) system. The MIT24 test involved examining the cells for morphological changes after 24 hours by optical microscopy and for changes in the color of phenol-red dye in the culture medium after 7 days. The data were compared with published results in other cell systems and of in-vivo studies where available. The concentrations causing 50% inhibition of metabolic activity (IC50s) generally compared favorably with cytotoxic endpoints in other in-vitro systems for nine compounds. The IC50s for 20 other compounds were generally well correlated with their median lethal doses following intraperitoneal injection in mice. The authors conclude that the mechanisms of in-vitro and in-vivo toxicity of the tested plasticizers appear to be similar. The lethality of the compounds to mice may reflect a basic cytotoxic effect on mouse tissues.

24
Shrivastava R, Delomenie C, Chevalier A, John G, Ekwall B, Walum E, Massingham R. COMPARISON OF IN VIVO ACUTE LETHAL POTENCY AND IN VITRO CYTOTOXICITY OF 48 CHEMICALS. Cell Biol Toxicol 1992;8(2):157-70.

The cytotoxicity of 48 compounds included in the MEIC

(Multicenter Evaluation of In Vitro Cytotoxicity) list was determined in cultures of rat hepatocytes, McCoy, and MDBK cells. The average minimum concentration of each compound inducing cytotoxicity was measured in each cell type. The cytotoxicity values were then compared with published oral LD50 values for rats and mice. The logarithmic transformation of in vivo toxic doses and the corresponding in vitro cytotoxic concentrations showed a statistically significant correlation between the in vitro and in vivo values. The results show that an accurate in vivo LD50 dose could be predicted from in vitro data for at least 75% of the selected compounds. It is hoped that this finding will not only stimulate others to pursue in vitro technique but will eventually lead to elimination of the in vivo LD50 test.

25
Oka M, Maeda S, Koga N, Kato K, Saito T. A MODIFIED COLORIMETRIC MTT ASSAY ADAPTED FOR PRIMARY CULTURED HEPATOCYTES APPLICATION TO PROLIFERATION AND CYTOTOXICITY ASSAYS. Biosci Biotechnol Biochem 1992; 56(9):1472-1473.

No abstract.

26
Klyuchareva TE, Matveeva VA, Kushlinskii NE. IN VITRO BIOASSAY FOR TYPE E PROSTAGLANDINS BASED ON THEIR NK IMMUNODEPRESSING ACTIVITY. Immunol Lett 1992; 33(3):239-45.

A biological assay for PGE in commercial preparations or secreted by tumor cells in culture fluid was developed on the basis of the immunosuppressive effect of PGE on the cytotoxic activity of NK cells. The assay is simple, rapid, and convenient for detecting PGE cell secretion, either spontaneous or induced by various signals. The sensitivity of the bioassay is limited by the sensitivity of NK cells to the immunosuppressive activity of PGE (i.e., about 10-8 M).

27
Grant RL, Yao C, Gabaldon D, Acosta D. EVALUATION OF SURFACTANT CYTOTOXICITY POTENTIAL BY PRIMARY CULTURES OF OCULAR TISSUES: I. CHARACTERIZATION OF RABBIT CORNEAL EPITHELIAL CELLS AND INITIAL INJURY AND DELAYED TOXICITY STUDIES. Toxicol 1992;76(2):153-76.

This investigation was undertaken to develop cytotoxicity assay systems using primary cultures of rabbit corneal epithelial cells as an experimental model to evaluate oculotoxic agents and the ability of these in vitro assay systems to predict irritancy potential and delayed toxicity. The authors have characterized the epithelial nature of the cultures by identifying keratins with antikeratin antibodies (AE1/AE3) and by demonstrating metabolic enzymes important to the integrity of the cell: lactate dehydrogenase, glucose 6-phosphate dehydrogenase and aldolase. Eight surfactants were compared and ranked according to their cytotoxic potential. The authors evaluated cytotoxicity by measuring leakage of the cytosolic enzyme, lactate dehydrogenase, into the medium, by making morphological observations and by assessing lysosomal neutral red uptake and mitochondrial 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reduction. The cells were treated for 1 h with the surfactants and the possibility of delayed toxicity was evaluated 24 h after removal of the surfactant. The cytotoxicity of the different types of surfactants as shown by all the tests was cationic > anionic = amphoteric > nonionic. The 24-h test for lactate dehydrogenase leakage showed that mild and nonirritating surfactants did not demonstrate any subsequent damage after a 1-h exposure, but the extreme and severe surfactants continued to showfurther damage after the 1-h exposure. In vitro findings were similar to reported in vivo results. In vitro cytotoxicity assays using primary cultures of rabbit corneal epithelial cells may be used to rank the cytotoxic potential of surfactants, but only the lactate dehydrogenase leakage test was able to assess prolonged cell injury.

28
Yao C, Acosta D. SURFACTANT CYTOTOXICITY POTENTIAL EVALUATED WITH PRIMARY CULTURES OF OCULAR TISSUES: A METHOD FOR THE CULTURE OF RABBIT CONJUNCTIVAL EPITHELIAL CELLS AND INITIAL CYTOTOXICITY STUDIES. Toxicol Methods 1992;2(3):199-218.

In order to rank the irritancy potential of chemicals objectively and biochemically, a primary culture method for rabbit conjunctival epithelial cells was developed as a potential in vitro method for ocular toxicity testing of xenobiotics. Conjunctival epithelial cells were dispersed by Dispase II, followed by trypsin

treatment. Cells were cultured in serum-free medium of 1:1 ratio of Dulbecco's modified Eagle's medium (DMEM) and F-12 nutrient mixture plus various concentrations of growth factors. At a plating d of 85,000 cells/cm2, cells grew to confluency in 3-4 days. Conjunctival cells showed a positive anti-keratin antibody stain which demonstrated their epithelial nature. These cells also showed positive periodic acid-Schiff (PAS) staining which is consistent with their goblet cell-containing and mucin-secreting function in vivo. Three surfactants, benzalkonium chloride (BzCl), sodium dodecyl sulfate (SDS), and Tween 20 (T-20; polyoxyethylene sorbitan monolaurate), at concentrations 2-20, 10-50, and 200-1500 mug/mL, respectively, were evaluated for their cytotoxicity potential. The results correlate well with reported results of the Draize eye irritancy test in vivo and suggest that a model of primary culture of rabbit conjunctival epithelial cells may be useful in predicting the eye irritancy potential of surfactants.

29
Ogawa Y, Murai T, Kawasaki H. MEDICAL DEVICES TESTING USING INTRACELLULAR ATP LEVEL OF ERYTHROCYTES AS AN INDICATOR FOR CYTOTOXICITY IN VITRO. Seitai Zairyo 1992;10(2):66-73.

The cytotoxicity of chemicals for erythrocytes can be detected by a simple, rapid, and inexpensive ATP assay. This ATP assay can be used for other mammalian cells.

30
Labrousse H, Pauillac S, Jehl-Martinez C, Legrand AM, Avrameas S. IN VIVO AND IN VITRO TECHNIQUES FOR THE DETECTION OF CIGUATOXIN. Oceanis 1991;18(2):189-91.

A review with 8 refs. Ciguatera illness is a food intoxication and can be provoked by eating fish from tropical coral reefs. Several techniques, briefly described here, are employed to detect toxic fish. The cat, the mongoose, or the young chick are used with in vivo techniques and are fed on samples with or without prepartion. Mice and mosquitoes are also used, but they require a more difficult extraction-purification phase. In vitro techniques call on immunochemical and the preparation of anti-ciguatoxin antibodies, as well as on physicochemical methods in which the preparation is made after adding the fluorescent group to purified ciguatera, and then using HPLC. Furthermore, a

cytotoxic method has been investigated. The detection limit required is very low, as man can already feel sick when concetrations range from 50 to 100 pg of ciguatera per g of fish flesh.

31
Chakravarty B, Srivastava S. TOXICITY OF SOME HEAVY METALS IN VIVO AND IN VITRO IN HELIANTHUS ANNUUS. Mutat Res 1992;283(4):287-294.

To compare the toxicity of some heavy metals in vivo and in vitro, the effects of six metals, aluminum (Al), cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn) were studied in the oil-yielding plant Helianthus annuus. The percentage of seed germination and cytotoxic effects at different concentra-tions and durations of treatment as well as the growth rate of callus tissues in vitro were compared to ascertain the concentrations that can either support plant growth or cause lethality. Highest toxicity to the plant system was observed from the effects of Pb both at high and low concentrations whereas Zn was the least toxic; and similar effects were seen in vivo and in vitro. The clastogenic effects of Al, Cd, Cu, and Ni were dependent on concentration and length of treatment. Cu and Zn showed less severe cytotoxic damages than Al, Cd, Pb, and Ni. In vitro growth could be supported at 100-1000 times the diluted concentrations of the metals in comparison to in vivo treatment.

32
Randall V, Mackay JM. A METHOD FOR EFFECTIVELY TESTING VOLATILE MATERIALS IN THE IN-VITRO CYTOGENETIC ASSAY IN HUMAN LYMPHOCYTES. Joint Meeting of the United Kingdom Environmental Mutagen Society/DNA Repair Network On Single Cell Gel Electorphoresis, Wales, England, UK, March 23-27, 1992. Mutagen 1992;7(5):384.

No abstract.

33
Babich H, Stern A, Munday R. IN VITRO CYTOTOXICITY OF METHYLATED PHENYLENEDIAMINES. Toxicol Lett 1992; 63(2):171-9.

The acute cytotoxicities of methylated phenylenediamines (PDs) were evaluated with the neutral red assay, using BALB/c 3T3 mouse fibroblasts as the

bioindicators. When the test agents were grouped according to their degree of methylation, good correlations were noted between their in vitro cytotoxicity and their in vivo myotoxicity to experimental animals, as well as to their in vitro autoxidation rates. For test agents of comparable methylation, the sequence of potency was ring-methylated p-PD > N-methylated p-PD > N-methylated o-PD > N-methylated m-PD.

DERMAL

34

Dick IP, Scott RC. THE INFLUENCE OF DIFFERENT STRAINS AND AGE ON IN VITRO RAT SKIN PERMEABILITY TO WATER AND MANNITOL. Pharm Res 1992;9(7):884-7.

Water and mannitol were used as test penetrants to study the effect of age on the skin permeability of the Wistar-derived Alderley Park (AP) rat and Sprague-Dawley (SD) rat. Whole-skin membranes were prepared from rats aged 10 to 120 days, while epidermal membranes were prepared from rats aged 24 to 32 days. The results indicated that the skin permeabilities of the two strains were very similar for either whole-skin or epidermal membranes. The influence of age on skin permeability was found to be negligible for the AP rat, and a small decrease in whole-skin permeability was observed for SD rats above 80 days of age. A statistically derived expression ("the separation efficiency factor") was used to determine the optimum age for preparing intact epidermal membranes; these were 26 days for AP rats and 28 days for SD rats. Dermal thickness, hair follicle depth, and, to a lesser extent, the surface area occupied by hair follicles all appeared to be influenced by age, although these changes had no detectable effect on skin permeability.

35

Barber ED, Teetsel NM, Kolberg KF, Guest D. A COMPARATIVE STUDY OF THE RATES OF IN VITRO PERCUTANEOUS ABSORPTION OF EIGHT CHEMICALS USING RAT AND HUMAN SKIN. Fundam Appl Toxicol 1992;19(4):493-497.

In vitro percutaneous absorption studies were carried out for eight chemicals using full thickness rat skin and human stratum corneum. The purpose of the studies was to compare the rates of absorption for the two species. For each of the chemicals, the observed rate using full thickness rat skin was greater than that

observed for human stratum corneum. The ratios of the rates (rat/human) varied from 1.7 to 5.8 with a mean value of 3.1. The chemicals tested were chosen to represent a wide range of physical properties and permeability constant values. It was concluded that rat skin was more permeable than human skin for each of these eight chemicals. The conclusion is supported by similar findings from studies in other laboratories and suggests that results from studies in the rat over estimate skin absorption in man.

36
Beele H, Thierens H, Deveux R, Goethals E, De Ridder L. SKIN ORGAN CULTURE MODEL TO TEST THE TOXICITY OF POLYOXYETHYLENE NETWORKS. Biomaterials 1992; 13(14):1031-1037.

Films of polyoxyethylene network were prepared from two types of triethoxysilane-terminated prepolymers. In this way, films of polyoxyethylene network with possible applications in the biomedical field could be made easier. To test their biocompatibility, these networks were added to organ cultures of adult human skin and embryonic chicken skin. A rapid toxic effect was observed, especially with the urethane-linked network. Enzymatical degradation of the network by enzymes in the culture medium might be responsible for the formation of toxic metabolites. Testing of related chemical compounds in our in vitro assay suggested that the formation of a silane group with an amino terminal is most likely to be responsible for the toxic effects observed.

37
Dick IP, Scott RC. PIG EAR SKIN AS AN IN VITRO MODEL FOR HUMAN SKIN PERMEABILITY. J Pharm Pharmacol 1992; 44(8):640-645.

Pig skin has been shown to have similar histological and physiological properties to human skin and has been suggested as a good model for human skin permeability. In this series of experiments, the in-vitro permeability of pig ear skin was compared with human (abdominal) skin and rat (dorsal) skin using both hydrophilic (water,mannitol, paraquat) and lipophilic (aldrin, carbaryl, fluazifop-butyl) penetrants. Pig skin was found to have a closer permeability character than rat skin to human skin, particularly for

lipophilic penetrants. Electrical conductivity measurements across pig skin membranes showed that skin conductivity could be a useful method for assessing the integrity of membranes, particularly when used in conjunction with water permeability assessments.

38
Berner B, Mazzenga GC, Gargiulo PM, Steffens R. TRANSDERMAL NICOTINE SYSTEM: FEASIBILITY STUDIES. J Controlled Release 1992;20(May):13-19. (33 REFS)

Nicotine diffusion through human epidermis in vitro, animal skin irritation studies, and the study of a membrane controlled rate limiting diffusion method for a transdermal system are discussed. Skin irritation in rabbits was shown to depend on the driving force of nicotine in the donor phase. A membrane-adhesive laminate was identified to provide substantial control and reduction of skin diffusion to minimize skin irritation.

39
Ridout G, Houk J, Guy RH, Santus GC, Hadgraft J, Hall LL. AN EVALUATION OF STRUCTURE-PENETRATION RELATIONSHIPS IN PERCUTANEOUS ABSORPTION. Farmaco 1992;47(6):869-92.

Prediction of chemical transport across skin is important both to the optimization of topical and transdermal drug delivery and to the assessment of risk following dermal exposure. To facilitate estimates of percutaneous absorption, a number of model in vitro experimental systems have been developed. However, the predictive applicability of the different approaches (with respect to human skin penetration), and the quantitative aspects of the structure-permeation behavior revealed, have not been critically evaluated. The objectives of this paper were to collect, from the literature, the more systematic investigations pertaining to chemical transport across the skin, to quantify the dependence of permeation on the lipophilicity of the penetrants studied, and to assess the relative utility of model systems for the prediction of percutaneous absorption. The experimental systems, used in the studies considered, involve, primarily, steady-state transport measurements across excised skin taken from either human cadavers or hairless mice. Favorable comparisons of these data to solute flux across simple organic liquid membranes are

possible. Overall, general patterns of behavior emerge from the analysis such that qualitative predictions can be made. From a quantiatiative standpoint, though, it is clear that additional "structure-activity" work is necessary to provide appropriate equations that can relate penetration between different test systems and between different chemical classes.

40
Ruland A, Kreuter J. INFLUENCE OF VARIOUS PENETRATION ENHANCERS ON THE IN VITRO PERMEATION OF AMINO ACIDS ACROSS HAIRLESS MOUSE SKIN. Int J Pharm 1992; 85(1-3):7-17.

The influence of various penetration enhancers including propylene glycol, oleic acid, Azone, iso-Pr myristate, valine, and nanoparticles on the permeation coefficients for the permeation of amino acids through hairless mouse skin as well as a dialysis membrane was assessed in vitro. The two different types of membranes were employed in order to distinguish between effects due to thermodynamic parameters and those due to barrier resistance. Furthermore, the influence of these penetration enhancers on the amount of amino acids remaining within the skin was determined. Oleic acid was the most efficient enhancer for amino acids [enhancement factor (EF) of 176 for histidine] followed by Azone (EF of 45 for phenylalanine). All other penetration enhancers failed to exert any significant effect on the skin permeation of amino acids. The fact that the enhancement effects of oleic acid and Azone are not reversible and that the enhancers exhibited no influence with dialysis membranes clearly indicate that both penetration enhancers induce their effects on the basis of changes in skin morphology.

DERMAL TOXICITY
41
Moody RP, Nadeau B, MacDonald S, Chu I. IN-VITRO SKIN ABSORPTION OF CARBON-14 CYANURIC ACID IN A SIMULATED SWIMMING POOL. Bull Environ Contam Toxicol 1993; 50(1):12-18.

No abstract.

42
Bason MM, Harvell J, Realica B, Gordon V, Maibach HI. COMPARISON OF IN VITRO AND HUMAN IN VIVO DERMAL

IRRITANCY DATA FOR FOUR PRIMARY IRRITANTS. Toxicol In Vitro 1992;6(5):383-387.

The value of the Skintex dermal assay system as a means of predicting the irritancy of four primary irritants at various concentrations was investigated. The four compounds tested were: benzalkonium chloride; hydrochloric acid, phenol, and trichloracetic acid. The results from the in vitro system were compared with published human in vivo data, which was obtained using 100 volunteers, and which graded the irritant reactions using a visual scale. The following parameters of the assay were determined: sensitivity, 82%; specificity, 71%; and positive predictive value, 82%. The in vivo dose-response curves for each of the four substances were compared with the in vitro dose-response curves, and correlation coefficients were calculated. The in vitro dose-response curves for benzalkonium chloride (r = 0.987) and phenol (r = 0.994) were strikingly similar to those generated in vivo, possibly indicating that the mechanisms of action in vivo and in vitro are similar for these two compounds. These studies should be extended to a broader variety of chemicals before unknown can be characterized with a reasonable degree of certainty using this in vitro method.

43
Gerberick GF, House RV, Fletcher ER, Ryan CA. EXAMINATION OF THE LOCAL LYMPH NODE ASSAY FOR USE IN CONTACT SENSITIZATION RISK ASSESSMENT. Fundam Appl Toxicol 1992;19(3):438-445.

The purpose of this study was to evaluate the utility of the murine local lymph node assay (LLNA) for contact sensitization risk assessment. Cellular proliferative activity in draining lymph nodes was determined for individual animals on Day 5 following four daily epicutaneous applications of the test chemical to the ears. Seventeen chemicals were tested, covering a range of materials including preservaties, drug actives, and perfume raw materials. The assay was found to be useful for identifying strong, moderate, and some weak sensitizers as defined by other testing methods (guinea pig, human). For evaluating the antigen specificity of the LLNA proliferative response, an in vitro blastogenesis assay was used. The results of the studies support the use of the murine LLNA for both investigative and predictive contact sensitization testing. The LLNA offers the advantages of requiring less time for completion, incorporating an objective

endpoint, requiring approximately half the number of animals, and being less costly than most currently employed guinea pig test methods. In addition, the authors believe the murine LLNA is a useful test to incorporate into a scheme for contact sensitization risk assessment. The major advantage of this approach is that the LLNA will provide information which will allow one to proceed directly to confirmatory human predictive testing without performing guinea pig testing.

44
Schwarzenbach R, Klecak G. DEVELOPMENT OF AN IN VITRO MODEL TO TEST THE METABOLISM OF SUBSTANCES IN THE SKIN. Parfuem Kosmet 1992;73(Jun):398-402.

The development of an in vitro skin model to accelerate and simplify the toxicological testing of new substances and significantly contribute to the reduction of animal experiments is described.

45
Li Q, Aoyama K. STUDY OF DOSE-RESPONSE RELATIONSHIP IN CONTACT SENSITIVITY USING AN IN VITRO ASSAY. Contact Dermatitis 1992;27(1):16-21. (22 REFS)

Dose response relationships in contact sensitivity were studied using an in-vitro assay. Hartley-guinea-pigs were sensitized with 1-chloro-2,4-dinitrobenzene (DNCB) by multiple subcutaneous injections at concentrations of 0, 10, 100 and 1,000 parts per million (ppm), with and without the addition of complete Freunds' adjuvant. This was followed 7 days later by the application of patches containing DNCB for 48 hours. Challenges with DNCB and 2,4-dinitrobenzene-sulfonic-salt (DNBS) were performed 14 days later using Finn Chambers. Lymph node cells were harvested after skin testing, and examined for transformation after the addition of various concentrations of DNBS and DNCB. Positive results to the DNCB challenge were seen in half of the animals given the low dose, and all of the animals treated with medium and high doses. Differences in the lymphocyte stimulation indices were seen between the control groups and the high and medium dose groups. Cross reactivity was seen in challenge and in-vitro tests using DNBS. A positive correlation scatter diagram was seen between stimulation indices and patch test readings challenged by DNCB, and a high degree of overlap of stimulation indices and patch score gradings

were seen as well.

46
King JR, Riviere JE, Monteiro-Riviere NA. CHARACTERIZATION OF LEWISITE TOXICITY IN ISOLATED PERFUSED SKIN. Toxicol Appl Pharmacol 1992; 116(2):189-201.

Lewisite (L) is a potent organic arsenical that causes rapid onset of pain and severe vesication on contact with epithelial tissues. The isolated perfused porcine skin flap (IPPSF) is an in vitro model that has shown potential as a model for cutaneous vesicant research. The objective of this study was to characterize IPPSF responses after topical exposure to six concentrations of L ranging from 0.07 to 5.0 mg/ml (n = 4/treatment plus controls). The sensitivity of the IPPSF to L exposure and the similarity of lesions to those described for humans suggests that this model provides a relevant in vitro model with which to study mechanisms of chemical vesication and arsenic toxicity, as well as protective and therapeutic intervention for vesicant exposure.

47
Basom M, Maibach HI. IN VITRO SKIN IRRITATION ASSAYS--CURRENT STATUS. Cosmet Toilet 1992; 107(Sept):67-68, 70. (17 REFS)

In vitro skin irritation assays for the toxicity testing of cosmetics without the use of animals are reviewed, and a list of in vitro irritation systems is presented.

48
Wasmus G, Bruckert J, Hamm B, Wodtke G, Weingaertner M, Schoene K, Schreiber G. DEVELOPMENT AND TESTING OF A METHOD FOR ASSESSMENT OF DERMAL EXPOSURE. Predict Percutaneous Penetration 1991;621-7.

The transdermal uptake of toxicants during work-place exposure is a sometimes underestimated problem. At present, with regard to German limits of work-place exposure, the transdermal risk is characterized more or less empirically. In order to obtain more information about the permeation of chemicals through skin, a routine in vitro test method is being developed for the Bundesanstalt fuer Arbeitsschutz, Dortmund, using pig

skin or polymer membranes instead of human skin. In order to obtain reliable information concerning permeation parameters for an assessment of transdermal toxicity risk, permeation rates and lag times can be evaluated by means of pig skin or PA11-based foils, preferably by gas-phase exposure.

49
Roguet R, Dossou KG, Rougier A. USE OF IN VITRO SKIN RECOMBINANTS TO EVALUATE CUTANEOUS TOXICITY: A PRELIMINARY STUDY. J Toxicol Cutaneous Ocul Toxicol 1992;11(4):305-315.

The cytotoxicity of surfactants (N cetyl-trimethylammonium bromide, sodium dodecyl sulfate, and sorbitan monooleate), formalin, and two non-water-soluble formulations was tested using a skin recombinant made of human keratinocytes cultured on dead de-epidermized dermis. Histologic observations and biochemical parameters of cytotoxicity (LDH release and MTT activity) are closely related. Moreover, as in the in vivo situation, cationic surfactant is more toxic than anionic and nonionic. Relative cytotoxicity of surfactants and formalin was markedly reduced on reconstructed epidermis in comparison with monolayer culture of human keratinocytes, emphasizing the toxicologic importance of the stratum corneum barrier. Histologic observations show that salicyclic acid, a well-known keratolytic agent in vivo, induced a reduction of the horny layer thickness of the skin recombinant. These preliminary results suggest that the skin recombinant on de-epidermized dermis may prove to be a useful in vitro model for the evaluation of cutaneous toxicity of topically applied substances.

50
Slivka SR, Zeigler F. USE OF AN IN VITRO SKIN MODEL FOR DETERMINING EPIDERMAL AND DERMAL CONTRIBUTIONS TO IRRITANT RESPONSES. J Toxicol, Cutaneous Ocul Toxicol 1993;12(1):49-57.

An in vitro skin model was used to study the responses of epidermal and dermal cells to irritants. This model consists of neonatal foreskin fibroblasts grown into a three-dimensional dermal structure on a nylon mesh. The dermal structure is seeded with keratinocytes and grown in calcium-contg. medium at the air-liq. interface until a fully differentiated epidermis was

formed. Phorbol-myristate acetate (PMA), a skin irritant, was incubated with this model. The PMA-conditioned culture medium was assayed for interleukin 1 alpha (IL-1 alpha) and tissue-type plasminogen activator (t-PA) by ELISA. Gelatin-contg. SDS-PAGE gels were used to measure prodn. of gelatinases. Exposure of the skin model to PMA resulted in the release of IL-1 alpha, t-PA, and gelatinases. Separation of the epidermis from the dermis and subsequent exposure of the dermis to PMA showed that in the presence of PMA the epidermis produces IL-1 alpha and the dermis alone produces t-PA. Both the epidermis and dermis produce gelatinases. Since IL-1 alpha is an important regulator of immune and inflammatory responses and t-PA and gelatinase activities are elevated in many skin disorders, it is believed that these assays in this in vitro skin model are useful for determining the contributions of epidermis and dermis to irritant responses.

51
Stafford RG, Mehta M, Kemppainen BW. COMPARISON OF THE PARTITION COEFFICIENT AND SKIN PENETRATION OF A MARINE ALGAL TOXIN (LYNGBYATOXIN A). Food Chem Toxicol 1992; 30(9):795-801.

Lyngbyatoxin A is produced by marine algae, and causes local cutaneous toxicity in swimmers. The purpose of this research was (1) to determine the partition coefficient of lyngbyatoxin A in octanol/water and (2) to use methods in vitro to measure the penetration and distribution of lyngbyatoxin A in guinea pig and human skin. Discs of excised guinea pig and human skin were mounted in diffusion chambers that exposed the epidermal surface to air and bathed the dermis with HEPES-buffered Hanks' balanced salt solution with gentamicin sulphate. HPLC was used to quantify lyngbyatoxin A. Skin penetration was calculated by summing the amount of lyngbyatoxin A recovered from the dermis and receptor fluid. The mean partition coefficient for lyngbyatoxin A was 1.53. Penetration of lyngbyatoxin A (expressed as a percentage of dose, n = 3) in guinea pig and human skin was 23 and 6.2 (respectively) after 1 hr of topical exposure. The amount of lyngbyatoxin A in the dermis and receptor fluid did not change significantly over time.

52
Lu MF, Lee D, Rao GS. PERCUTANEOUS ABSORPTION

ENHANCEMENT OF LEUPROLIDE. Pharm Res 1992; 9(12):1575-9.

Chemical enhancers and vehicles were tested for their ability to improve the percutaneous absorption of leuprolide, a nonapeptide (LH releasing hormone analog). In vitro permeabilities in nude mouse, snake, and cadaver skin were evaluated in either Franz diffusion cells or a Bonaugh flow-through system using an HPLC assay. Skin irritation caused by the formulations was evaluated in the rabbit. The chemical enhancer systems investigated strongly enhanced skin penetration of leuprolide. The in vitro permeability in nude mouse skin was 10 or 100 times higher than that obtained in cadaver skin, depending on the type of enhancer that was used in the formulation. Snake skin was at least 10 times less permeable than cadaver skin in this study. However, the effects of chemical enhancers on skin permeability were highly dependent on the skin model. Further, the in vitro permeability of leuprolide in the base form was 10 times higher than in the acetate form with the enhancers.

53
Mori S, Nishimura N, Nakamura T, Masuda M, Oba K. THE LYMPHOCYTE PROLIFERATION ASSAY AS AN IN VITRO ALTERNATIVE METHOD TO SENSITIZATION TESTS. In Vitro Toxicol;5(3):147-60.

This report describes an antigen-specific lymphocyte proliferation assay for use as a screening method to detect skin sensitizers. Mice were immunized by topical application of sensitizers. Five days later lymphocytes were collected from the draining lymph nodes and stimulated in vitro with the immunizing sensitizer. After optimization of the assay procedures, a substantial proliferative response was observed to known water-solution and insoluble strong sensitizers (p-phenylene-diamine, fluorescein isothiocyanate, dinitrochlorobenzene, N,N-dimethyl-p-nitroso-aniline, oxazolone). The stimulation index (sensitizer-specific response vs. background response) increased dramatically using fresh homologous mouse serum. By this method the antigenicity of weaker sensitizers (glutaraldehyde, cinnamic aldehyde and neomycin) could be detected. This rapid and simple assay could be used to prescreen sensitizers.

EMBRYOTOXICITY

54

Tempel KH, Ignatius A, Stammberger I. A SHORT-TERM TEST FOR NUCLEOTOXICITY THAT USES CHICK EMBRYO CELLS TREATED IN VITRO AND IN VIVO--PHYSICO-CHEMICAL AND BIOCHEMICAL INVESTIGATIONS. Comp Biochem Physiol [C] 1992; 103(1):73-8.

1. In vitro, some nucleotoxic or potentially nucleotoxic agents were tested with freshly isolated liver and brain cells from chick embryos as well as with thymic cells of the rat. 2. In vivo, chicken embryos were exposed to X-rays, methyl methanesulfonate, methyl nitrosourea, triethylene thiophosphoramide, and dimethylnitrosamine. 3. The toxic effects were determined by viscometry of alkaline cell lysates, nucleoid sedimentation, scheduled (SDS) and unscheduled (UDS) DNA synthesis and/or RNA synthesis. 4. The dose-effect curves obtained in vitro show that directly acting genotoxic agents are detected by the embryonic cells with equal or comparable sensitivity as by mammalian cells. 5. In vivo, genotoxicity is reflected by a decrease in alkaline lysate viscosity, nucleoid sedimentation and SDS and an increase in UDS. 6. From the present results it is suggested that chick embryo cells offer a simple, rapid and inexpensive short-term nucleotoxicity test for directly acting agents, the main disadvantage being the innate inability to biotransform indirectly acting agents.

55

Tsuchiya T, Eto K, Burgin H, Kistler A. MICROMASS CULTURE OF MIDBRAIN CELLS AND ITS RELEVANCE TO IN VITRO MECHANISTIC STUDIES. Congenital Anom 1992;32(2):105-116.

The relationship between ethylenethiourea (ETU)-induced malformations of cultured rat whole embryos and the alterations of midbrain (MB) cells was investigated and species-specific ETU-induced alterations between rat and mouse MB cells were determined. Serum samples were prepared from rats and mice given ETU, and ETU-teratogenicity was evaluated in both species. We determined that the different sensitivity of the midbrain of the rat and mouse may be the main reason that ETU was teratogenic in rats but not in mice. Next, the authors showed that MB-cultures are unsuitable for estimating the teratogenic potential of arotinoids. MB differentiation was adversely affected only at concentrations which caused cell death. Finally, it

was demonstrated that the embryolethal action of new herbicides is not detectable in the micromass teratogenic test. However, it was concluded that the V79 colony assay may be useful for preliminary screening of embryolethal effects of herbicides.

56
Farage-Elawar M, Rowles TK. TOXICOLOGY OF CARBARYL AND ALDICARB ON BRAIN AND LIMB CULTURES OF CHICK EMBRYOS. J Appl Toxicol 1992;12(4):239-244, 27.

Brain cells and limb cells harvested from 5 day old chick embryos were exposed to aldicarb (116063) (40 to 200 parts per million (ppm)) or carbaryl (63252) (5 to 60ppm). Carbaryl without activation was extremely toxic to brain cells and a significant reduction in brain cell neutral-red elution was noted at 15 to 60ppm exposure. With S-9 activation, the 40 and 60ppm levels significantly reduced the neutral-red elution of brain cells, causing 67 and 50% viability of the acetone control, respectively. Aldicarb without S-9 caused a significant increase in neutral-red elution; with S-9 addition the number of cells was near 100% the acetone control. Carbaryl without in-vitro activation was significantly toxic to limb cultures at 8 to 25ppm levels. Aldicarb alone had no effect on the limb cultures, but when S-9 was added the highest two doses caused a significant reduction in the number of limb cells. Carbaryl alone significantly affected the area of the colonies. The findings demonstrated that chick embryo cultures of brain and limb cells could be used as in-vitro toxicological assay systems. Assay benefits included ease of reproduction and maintenance and lack of expense.

GENOTOXICITY
57
Shaddock JG, Feuers RJ, Chou MW, Pegram RA, Casciano DA. EFFECTS OF AGING AND CALORIC RESTRICTION ON THE GENOTOXICITY OF FOUR CARCINOGENS IN THE IN VITRO RAT HEPATOCYTE/DNA REPAIR ASSAY. Mutat Res 1993;295(1):19-30.

The effects of aging and chronic caloric restriction (CR) on the genotoxicity of four carcinogens, representing four different classes of chemicals, in the in vitro rat hepatocyte/DNA repair assay were investigated. Hepatocyte cultures were isolated from young, middle-aged, and old male Fischer (F344) rats which were maintained on either an ad libitum (AL) or a

CR diet (60% of AL). Hepatocyte cultures from old AL rats, treated with 2-acetyl-aminofluorene (2-AAF), aflatoxin B1 (AFB1), 7,12-dimethyl-benz[a]anthracene (DMBA) and dimethylnitrosamine (DMN), exhibited age-related decreases in DNA repair as compared to young AL rats. Also, cultures from young CR rats exhibited significant diet-related decreases in DNA repair with 2-AAF, AFB1, DMBA and DMN, when compared to results from young AL diet-fed rats. The data indicate an age- and diet-related decrease in DNA repair and/or DNA damage and suggest that this decrease is due to a decrease in metabolic activation of these carcinogens to genotoxic species.

58
Oesch F, Oesch-Bartlomowicz B, Arens HJ, Friedberg T, Utesch D, Glatt HR, Platt KL. MOLECULAR AND CELLULAR BASIS FOR ADEQUATE METABOLIC DESIGN OF GENOTOXICITY STUDIES. Toxicol Lett 1992;64-65:643-9. (12 REFS)

Genotoxic species and metabolites are usually under the control of a complex set of activating, inactivating and precursor sequestering enzymes. These enzymes differ greatly between test systems, animal species and man. An adequate metabolic design of genotoxicity studies requires careful attention to factors such as: dilution of cofactors in in vitro tests which are present in much higher concentrations in the intact cell; induction in high dose carcinogenicity bioassays of enzymes, which are constitutively not expressed and not induced at such doses of the compound, which occur in the situations of the practical use of the compound; modifications of control enzymes, which are effected by hormones or other endogenous factors, which are differently influenced by high dose (bioassay) vs. moderate dose (real exposure) or by in vivo (endocrine regulation) vs. in vitro (no endocrine regulation) conditions.

59
Schmezer P, Kuchenmeister F, Pool-Zobel BL. MICROGEL SINGLE CELL ASSAY A NOVEL TECHNIQUE TO DEMONSTRATE GENOTOXICITY IN SOMATIC MAMMALIAN CELLS. Joint Meeting of the United Kingdom Environmental Mutagen Society/DNA Repair Network on Single Cell Gel Electrophoresis, Wales, England, UK, March 23-27, 1992. Mutagenesis 1992;7(5):384.

No abstract.

60
Baker RSU, Bonin AM, Arlauskas A, He S, Coombs MM.
TUMORIGENICITY OF CYCLOPENTA(A)PHENANTHRENE DERIVATIVES AND MICRONUCLEUS INDUCTION IN MOUSE SKIN.
Carcinogenesis 1992;13(3):329-332. (17 REFS)

A comparison was made of the abilities of 15,16-dihydro-11-methylcyclopenta(a) phenanthrene-17-one and its unmethylated parent compound to induce micronuclei in epidermal keratinocytes following their application to the skin of Skh/HR-1-hairless- mice. Only the 11-methyl derivative was observed to be carcinogenic in this study. A preliminary study was then performed to determine the doses of each chemical that allowed cell survival for micronucleus induction. A correlation was demonstrated between micronucleus induction in epidermal keratinocytes and the cancer initiating potential of the two compounds. A statistically significant increase in micronuclei over the range of 10 to 100 nanomoles was noted only on exposure to the carcinogenic compound, the 11-methyl derivative. The authors stress that the skin micro-nucleus method has been demonstrated to provide a useful means of examining the genotoxicity and carcinogenicity relationship in the same target tissue in-vivo and it also allows a comparison to be made between the in-vitro and in-vivo genotoxicity of a given compound.

61
Brice AJ. THE USE OF CULTURED HUMAN LYMPHOCYTES TO ASSESS GENOTOXICITY. SCI (Society of Chemical Industry) Pesticides Group Symposium on In Vitro Systems for Testing Activity, Toxicity and Metabolism, London, England, UK, November 4, 1991. Pestic Sci 1992;35(3): 293-294.

No abstract.

62
Bajrakova A. SOME NEW TRENDS IN THE STUDIES OF MUTAGENICITY AND CARCINOGENICITY. Probl Nukl Med Radiobiol Radiatsionnata Khigiena 1991;12(0):74-80.

In the investigations for mutagenicity and carcinogenicity, short-term tests are used for genotoxicity, longer-term experiments on animals and epidemiologic studies of people. The information,

obtained by short-term tests for genotoxicity, underlies the computer programms, which follow two trends: for optimal supplying of tests and for predicting the relationship between the chemical structure and the mutagenic and the carcinogenic potential of the agent. The possibilities and limitations of the molecular dosimetry are discussed in view of the extrapolation of the results, obtained from in vitro and in vivo studies and the biomonitoring of the populations at risk. The new trends are presented of the epidemiologic studies for the congenital lesions, related with the use of definite indices (sentinelle phenotypes, inborn malformations, etc.) and the perspectivity of the methods, which allow the demonstration of definite mutations not only in the somatic cells, but also in the mature male gametes (spermatozoa) in man.

63
Hellmer L, Bolcsfoldi G. AN EVALUATION ON THE ESCHERICHIA COLI K-12 UVRB/RECA DNA REPAIR HOST-MEDIATED ASSAY: I. IN VITRO SENSITIVITY OF THE BACTERIA TO 61 COMPOUNDS. Mutat Res 1992; 272(2):145-160.

A differential DNA repair test was evaluated in vitro, using derivatives of Escherichia coli K-12 343/113 with the genotype uvrB-/recA- and uvr B+/recA+. The aim of this study was to characterize the sensitivity of the assay to different compounds in vitro and thereby provide information on the usefulness of this end-point as an indicator of genotoxicity in a host-mediated assay. Sixty-one compounds from diverse chemical groups were tested and of these 32 gave a positive result. The results obtained compared with results from the Ames test and were in agreement for 49 out of the 61 compounds tested. Chemicals that were detected in this test but negative in the Ames test were 4-aminophenol, catechol, diethylstilbestrol, thioacetamide and thiourea. Seven of the compounds tested gave a negative result in E. coli but were positive in Salmonella assays. These were 4-aminobiphenyl, benzo(a)pyrene, cyclophosphamide, 1-naphthylamine, N-nitrosobutylpropylamine, quinoline and 2-toluidine. The performance of the in vitro test and reasons for the discrepant results with the Ames test are discussed. The overall concordance between the two tests was about 80%. On the basis of these results we consider these bacterial strains, and differential DNA repair as an end-point, to be sufficiently accurate as

an indicator of genotoxicity in vitro and thereby also in vivo.

64
Jung R, Steinle D, Anliker R. A compilation of genotoxicity and carcinogenicity data on aromatic aminosulfonic acids. Food Chem Toxicol 1992; 30(7):635-60.

A review with many references on genotoxicity and carcinogenicity testing of various aromatic aminosulfonic acids (AASAs). Comparisons were made with the data available on the corresponding unsulfonated analogs, some of which are known to be genotoxic and(or) carcinogenic. The vast majority of the AASAs were conclusively nonmutagenic in the Ames test. In most cases, the absence of genotoxicity was also demonstrated with a variety of other test systems in vitro and in vivo. Evidently, AASAs in contrast with some of their unsulfonated analogs generally have no or very low genotoxic and tumorigenic potential.

65
Matsuoka A, Yamazaki N, Suzuki T, Hayashi M, Sofuni T. EVALUATION OF THE MICRONUCLEAR TEST USING A CHINESE HAMSTER CELL LINE AS AN ALTERNATIVE TO THE CONVENTIONAL IN VITRO CHROMOSOMAL ABERRATION TEST. Mutat Res 1992; 272(3):223-36.

The in vitro micronucleus (MN) test was carried out simultaneously with the conventional chromosomal aberration (CA) test on 11 clastogenic chemicals or spindle poisons with different modes of action using a Chinese hamster cell line (CHL). The method of slide preparation for the MN test was the same as that for the conventional metaphase analysis, except that 1% acetic acid in methanol was used as the cell suspension medium for air-drying (to preserve the cytoplasm around the nucleus). All chemicals tested induced micronuclei reproducibly and dose-dependently in good agreement with the results of metaphase analysis. Since the MN test methodology is simple and the observation of MN is less subjective than that of CA, the authors conclude that the in vitro MN test would be a good alternative to the conventional CA test for screening the genotoxicity of chemicals.

66

Nieboer E, Rossetto FE, Turnbull JD. MOLECULAR BIOLOGY APPROACHES TO BIOLOGICAL MONITORING OF GENOTOXIC SUBSTANCES. Toxicol Lett 1992; 64-65 Spec No:25-32. (33 REFS)

Genetic testing is subdivided into genetic monitoring (evaluation over time of induced genetic changes) and genetic screening (detection of inherited traits). Genetic factors in relation to susceptibility to environmental agents are briefly examined, as well as mutation assays suitable for use in genetic monitoring, techniques for identifying specific DNA lesions, and oncogene products as biomarkers. In vitro studies with AS52 Chinese hamster ovary cells indicate that the distribution of lesions (e.g., point mutations or segment deletions) at the xanthine-guanine phosphoribosyl transferase (gpt) gene in mutants generated by exposure to nickel compounds show some substance specificity. This ability is viewed as a promising development for the molecular epidemiology of occupational and environmental cancers. It is concluded that technical limitations pertaining to specificity and sensitivity, as well as ethical and legal implications, need to be resolved before routine application of genetic monitoring and screening is feasible.

67
Allavena A, Martelli A, Robbiano L, Brambilla G. EVALUATION IN A BATTERY OF IN VIVO ASSAYS OF FOUR IN VITRO GENOTOXINS PROVED TO BE NONCARCINOGENS IN RODENTS. Teratogenesis, Carcinogenesis, and Mutagenesis 1992;12(1):31-41. (27 REFS)

The genotoxic effects of 2-chloroethanol, 8-hydroxyquinoline, 2,6-toluenediamine, and eugenol were studied in Sprague-Dawley rats. Animals were treated with half of the 50% lethal dose of each substance by gavage. One group was treated 20 hours after partial hepatectomy, and liver and bone marrow cells examined after 48 hours; another group was treated 30 and 6 hours prior to sacrifice, and bone marrow cells examined and primary cultures prepared from the liver; and a third group was sacrificed 2 hours after treatment and hepatocyte primary cultures prepared. No increases in the appearance of micronucleated hepatocytes were seen in any treated animals after partial hepatectomy. No increases in the numbers of micronucleated polychromatic erythrocytes, or changes in the frequencies of polychromatic

erythrocytes were seen in the bone marrow of animals in either of the first two experimental groups. No significant increases in unscheduled DNA synthesis were seen in the third group of animals upon autoradiographic evaluation of hepatocyte primary cultures, and no evidence of DNA fragmentation was found. The authors conclude that contrary to their actions in in-vitro carcinogenicity tests, the tested compounds do not produce significant in-vivo genotoxic effects.

68
Brendler SY, Tompa A, Hutter KF, Preussmann R, Pool-Zobel BL. IN VIVO AND IN VITRO GENOTOXICITY OF SEVERAL N-NITROSAMINES IN EXTRAHEPATIC TISSUES OF THE RAT. Carcinogenesis 1992;13(12):2435-41.

Toxicological mechanisms involved in organotropism of tumor induction may include cell-specific metabolic activation of the carcinogen, in vivo distribution of active metabolites and persistance of induced DNA damage. In order to elucidate which factors are involved in the organotropic action of environmentally relevant N-nitrosamines, the authors have studied thier genotoxic and cytotoxic effets within primary intact cells of lung and kidney. The end-points determined were cytotoxicity by trypan blue exclusion and DNA single-strand break (SSB) induction by alkaline filter elution. The assays were performed in vitro to determine organ-specific metabolic activation by incubating the celss with the test compounds.

HEPATOTOXICITY
69
DelRaso N. IN VITRO METHODS FOR ASSESSING CHEMICAL OR DRUG TOXICITY AND METABOLISM IN PRIMARY HEPATOCYTES. In Vitro Methods Toxicol 1992;175-201. (94 REFS)

No Abstract.

IMMUNOTOXICITY
70
Krieger J, Fletcher R, Neblock D, Michini P, Siegel S, Taylor R, Daddona P. A NOVEL IMMUNOASSAY FOR THE DETECTION OF ENDOTOXIN-ANTI-ENDOTOXIN ANTIBODY IMMUNE COMPLEXES. 32nd Interscience Conference on Antimicrobial Agents and Chemotherapy, Anaheim, California, USA, October 11-14, 1992. Program Abstr

Intersci Conf Antimicrob Agents Chemother 1992; 32(0):307.

No abstract.

71
Mori S, Nishimura N, Nakamura T, Masuda M, Oba K. THE LYMPHOCYTE PROLIFERATION ASSAY AS AN IN VITRO ALTERNATIVE METHOD TO SENSITIZATION TESTS. In Vitro Toxicol 1992;5(3):147-160.

This report describes an antigen-specific lymphocyte proliferation assay for use as a screening method to detect skin sensitizers. Mice were immunized by topical application of sensitizers. Five days later lymphocytes were collected from the draining lymph nodes and stimulated in vitro with the immunizing sensitizer. After optimization of the assay procedures, a substantial proliferative response was observed to known water-soluble and insoluble strong sensitizers. Water-insoluble sensitizers were solubilized in appropriate solvents. In order to increase the sensitivity of the assay, homologous serum (fresh mouse serum) was used in the lymphocyte culture medium instead of heterologous serum (horse serum and fetal calf serum). It was found that the stimulation index (sensitizer-specific response versus background response) increased dramatically using the homologous serum. It is therefore considered that this rapid and simple assay could be used to prescreen sensitizers.

72
Pallardy M, Lebrec H, Blot C, Burleson GR, Bohuon C. NEW METHODS FOR THE ASSESSMENT OF IMMUNOTOXICITY OF CHEMICAL SUBSTANCES. Health Effects Research Lab., Research Triangle Park, NC, USA. Govt Reports Announcements & Index (GRA&I), Issue 24, 1992.

The immune system is a target of toxic insult following subchronic or chronic exposure to environmental chemicals, therapeutic drugs or abused drugs. Interaction of xenobiotics with the immune system may result in undesirable effects of three principal types: (1) those manifested as immunosuppression, (2) those manifested as autoimmunity, and (3) those manifested as an allergic reaction. Additional qualitative and quantitative information should improve the accuracy with which the effects of chemicals and drugs on the immune system can be predicted. To extend and develop

the approach the authors have focused on in vitro

systems. In vitro animal systems are potent tools to permit identification of a chemical's effect on isolated parts of the immune system and results may be correlated with human peripheral blood lymphocytes. The present study evaluated the in vitro effects of a panel of selected drugs on the immune system to compare and validate the assays employed and to determine the sensitivity of the methods used.

73
Bessler WG. SYNTHETIC LIPOPEPTIDE IMMUNOMODULATORS DERIVED FROM BACTERIAL LIPOPROTEIN: TOOLS FOR THE STANDARDIZATION OF IN VITRO ASSAYS. Dev Biol Stand 1992;77:49-56.

For the evaluation of immunomodulators by in vitro assays, agents that work reproducibly are difficult to obtain; conventional preparations of bacterial immunomodulators tend to vary for different preparations. The authors suggest that synthetic lipopeptide analogues derived from bacterial lipoprotein may be used as standards for various in vitro assays: studying B lymphocyte activation, lipopeptides acting as potent mitogens and polyclonal activators inducing immunoglobulin synthesis. In monocytes/ macrophages, lipopeptides stimulate the secretion of IL-1, IL-6, tumor necrosis factor (TNF) and nitrogen oxide (NO); they also induce tumor cytotoxicity. Lipopeptides also constitute potent immuno-adjuvants in vitro and in vivo, either in combination with or covalently bound to antigen. The novel synthetic lipopeptides described here can be synthesized readily in gram amounts with high purity and reproducibility; they are non-toxic and can be stored for a long time even at room temperature. Thus, lipopeptides meet the requirements to serve as effective standards for a multitude of relevant biological assays.

MEMBRANE TOXICITY
74
Walum E. MEMBRANE LESIONS IN CULTURED MOUSE NEUROBLASTOMA CELLS EXPOSED TO METAL COMPOUNDS. Toxicol 1982;25(1):67-74. (26 REFS)

A proposed method for screening heavy metal compounds for their membrane toxicity was developed. The method

was based on preloading cultures of clone-41A3 mouse-neuroblastoma-C1300 cells with tritium (H-3) tagged 2-deoxy-D-glucose (dGlc). The cultures were perfused with phosphate buffered saline containing 1.0mg/ml D-glucose and various concentrations of the test compound for 60 minutes. Efflux of dGlc derived H3 activity into the perfusate which served as a marker for membrane damage was monitored. The membrane toxic concentration (MTC) was computed from the data. The technique was tested with up to 10(-3) molar (M) mercuric-chloride, methylmercuric-chloride (MMC), triethyltin-chloride (TETC), and potassium-dichromate. The MTCs of the compounds were: MMC, 9x10(-7)M; mercuric-chloride, 6x10(-6)M; TETC, 3x10(-4)M; and potassium-dichromate, 7x10(-4)M. The author concludes that ranking the membrane toxicity of the compounds according to their MTCs indicates that MMC is the most toxic followed by mercuric-chloride, TETC, and potassium-dichromate in that order. The ranking agrees with the results of previously published studies. This indicates that the proposed bioassay can be used to screen metal compounds for their nerve cell membrane toxicity.

MUTAGENESIS

75

Thilly WG. COMPARATIVE MUTAGENESIS OF HUMAN CELLS IN VIVO AND IN VITRO. Department of Energy, Washington, DC. Govt Reports Announcements & Index (GRA&I), Issue 24, 1992.

This report discusses measuring methods of point mutations; high density cell cultures for low dose studies; measurement and sequence determination of mutations in DNA; the mutational spectra of styrene oxide and ethylene oxide in TK-6 cells; mutational spectrum of Cr in human lymphoblast cells; mutational spectra of radon in TK-6 cells; and the mutational spectra of smokeless tobacco. Sponsored by Department of Energy, Washington, DC.

76

Hozier J, Applegate M, Moore MM. IN VITRO MAMMALIAN MUTAGENESIS AS A MODEL FOR GENETIC LESIONS IN HUMAN CANCER. Mutat Res 1992;270(2):201-9.

Molecular and cytogenetic analyses of mutations induced by a variety of genotoxic compounds at the heterozygous thymidine kinase locus in mouse lymphoma cells indicate

that this in vitro assay does indeed register the range of genetic lesions recently found in a wide variety of human tumors. The types and complexity of the induced lesions are reflected in mutant colony phenotype in a compound-specific fashion. These studies point to the use of appropriate in vitro mammalian mutagenesis assays as new model systems for dissecting the genetic lesions important in human carcinogenesis, and as a means of determining the potential for compounds to induce such lesions.

77
Benigni R. RELATIONSHIPS BETWEEN IN VITRO MUTAGENICITY ASSAYS. Mutagenesis 1992;7(5):335-41.

This paper analyzes the mutagenicity results reported by the US National Toxicology Program (NTP), relative to 41 chemicals assayed with four in vitro short-term tests [Salmonella typhimurium (STY), Chromosomal aberrations in Chinese hamster ovary (CHO) cells (CHA), Sister chromatid exchange in CHO cells (SCE), mutation in L5178Y mouse lymphoma cells (MLY)] and puts this database in perspective with respect to other databases. It is shown that the test relationships pointed out by the experiments on the 41 chemicals are in substantial agreement with those indicated by a previous NTP report on 73 chemicals, and that the same test relationships were also indicated by the results on the International Program for the Evaluation of Short-Term Tests for Carcinogens (IPESTTC). The NTP and IPESTTC databases consistently indicated that there is a gradual increase in the sensitivity to the genotoxins in the following order: STY < CHA < SCE < MLY. A mathematical simulation analysis demonstrated that MLY and SCE--the two most sensitive assays of those studied by NTP--are not more subject to erratic results than other assays, and that they form--together with STY and CHA--a consistent family of genotoxicity assays.

NEPHROTOXICITY
78
Sina JF, Bradley MO. AN IN VITRO NEPHROTOXICITY ASSAY IN RENAL PROXIMAL TUBULES. In Vitro Methods Toxicol 1992;81-91.

A review with 38 refs. which focuses on the utility and limitations of renal tubule suspensions in toxicology. Various points to consider in applying the in vitro

model to studies of nephrotoxicity are emphasized.

79
Trevisan A, Meneghetti P, Masao S, Secondin L, Nicoletto G. SEX-AND AGE-RELATED NEPHROTOXICITY DUE TO 1,2-DICHLOROPROPANE IN VITRO. Arch Toxicol 1992; 66(9):641-645.

Sex- and age-related nephrotoxicity due to 1,2-dichloropropane was studied in vitro by means of renal cortical slices obtained from Wistar rats. Reduced glutathione content, organic anion accumulation (p-aminohippurate), and release of malondialdehyde (to measure the extent of lipid peroxidation), aspartate aminotransferase, gamma-glutamyltransferase and lactate dehydrogenase into the incubation medium were determined. Sex differences in naive rat parameters were slight, but male were more susceptible to toxic effects of 1,2-dichloropropane than female rats; glutathione depletion, lipid peroxidation, and loss of organic anion accumulation were higher in male than in female slices. During senescence, naive male rats showed a progressive decrease of glutathione content (statistically significant from 7-9 months of age), increase of spontaneous lipid peroxidation from the same age, and increase of signs of cytotoxicity (release of aspartate aminotransferase and lactate dehydrogenase into the incubation medium) from 3-4 months of age. Slices from rats of 3-4 months old showed the apparently highest susceptibility to 1,2-dichloropropane but depletion of glutathione content and loss of organic anion accumulation were at the same level in the oldest rats. The age decrease of control values caused the differences in the percentage ratio and then, apparently, a lower DCP effect. On the contrary, the increase of aspartate aminotransferase released in the incubation medium by DCP-treated slices corresponded to the age-related increase in cytotoxicity.

NEUROTOXICITY
80
Brat DJ, Brimijoin S. A PARADIGM FOR EXAMINING TOXICANT EFFECTS ON VIABILITY, STRUCTURE, AND AXONAL TRANSPORT OF NEURONS IN CULTURE. Mol Neurobiol 1992; 6(2-3):125-35. (51 REFS)

N1E.115 murine neuroblastoma cells differentiating in serum-free medium were used to develop a paradigm for

testing neurotoxicity in vitro. The paradigm was designed to test the effects of toxicants on four different aspects of cell function or structure: 1. Viability as shown by the retention of cellular radiolabel (51Cr); 2. Growth and maintenance of neurites as reflected by the incidence and average length of these processes; 3. Gross structure of neurites; and 4. Velocity and flux of rapid anterograde and retrograde axonal transport as judged by video-enhanced differential interference contrast microscopy. To evaluate this paradigm, colchicine and vinblastine were used as neurotoxicants with a well-understood mechanism of action. These agents were only weakly cytotoxic according to the Cr-release assay, but were able to interfere with neurite outgrowth at nanomolar concentrations. Neurites that were elaborated in the presence of vinblastine and colchicine were often disfigured by numerous swellings packed with organelles. In established neurites, micromolar concentrations of vinblastine inhibited organellar motility with great rapidity, blocking all signs of transport within 20 min. The effect of colchicine was slower and less complete, but still impressive. The results suggest that this four-part analysis represents a highly sensitive in vitro test for neurotoxicity, and a means of analyzing the relation between abnormalities of transport and structural damage of nerve cells.

81
Claudio L. AN ANALYSIS OF THE U.S. ENVIRONMENTAL PROTECTION AGENCY NEUROTOXICITY TESTING GUIDELINES. Regul Toxicol Pharmacol 1992;16(2):202-12. (67 REFS)

Few of the more than 65,000 chemicals listed in the Environmental Protection Agency (EPA) inventory have been tested for neurotoxicity. The nervous system may be especially vulnerable to toxicants because many compounds can cross the blood-brain barrier and induce irreversible damage. Additionally, the young, the elderly, and other sensitive populations may be particularly susceptible to neurotoxic injury. The EPA has developed guidelines including neurobehavioral, neuro-pathological, and neurochemical tests for the identification of possible neurotoxicants. In the present review, tests included in the current EPA guidelines for neurotoxicity testing are described and evaluated. Validation data on these tests are available for many known neurotoxicants. It is suggested that alternative tests be considered for

screening of large numbers of chemicals and that testing priority be given to chemicals on the basis of structure/activity relationships, lipophilicity, bioaccumulation, and extent of exposure.

82
Veronesi B. IN VITRO SCREENING BATTERIES FOR NEUROTOXICANTS (JOURNAL ARTICLE). Govt Reports Announcements & Index (GRA&I), Issue 21, 1992

The need to develop, validate and utilize in vitro models to test chemicals for neurotoxic potential is widely appreciated. The report discusses the major advantages of using cell and tissue culture, the various in vitro models amenable for neurotoxicity studies, and the distinction between mechanistic and screening models. Considerations for designing screening batteries to evaluate neurotoxicants are discussed. Topics such as choice of appropriate cell models and endpoints (i.e. cytotoxic and neurotoxic), and technical considerations in the design of the battery are also presented.

83
Nostrandt AC, Rowles TK, Ehrich M. CYTOTOXIC EFFECTS OR ORGANOPHOSPHORUS ESTERS AND OTHER NEUROTOXIC CHEMICALS ON CULTURED CELLS. In Vitro Toxicol 1992; 5(3):127-136.

The capability of a neuronal cell line to respond to 5 neurotoxic chemicals was assessed by using alterations in viability, morphology, acetylcholinesterases (AChE) activity, and free intracellular calcium ion concentration ((Ca2+)i) as indices of cytotoxicity. Differentiated SY-5Y cells were used as the test system for examination of these effects, as they were particularly useful for the study of early effects of a neuropathy-inducing organophosphate (OP), mipafox. Other similar compounds were studied for their cytotoxic effects in SY-5Y cells. Results indicated that acetylcholinesterase inhibition was the most sensitive indicator of neurotoxicity of the OPs and the carbamate. Inhibition of AChE was observed within 10 minutes of toxicant exposure and at concentrations of these compounds that did not affect viability at 24 hours. Concentrations that affected AChE within minutes did not affect morphology or (Ca2+)i until hours or days later. For compounds that did not have as notable an effect on AChE (IDPN and carbachol),

continuous exposures over several days were needed before cytotoxic effects were noted. The results indicate that toxic effects of esterase inhibitors may be more easily detected in the differentiated SY-5Y cell culture system than toxic effects of other chemicals.

84
Ali SF, LeBel CP, Bondy SC. REACTIVE OXYGEN SPECIES FORMATION AS A BIOMARKER OF METHYLMERCURY AND TRIMETHYLTIN NEUROTOXICITY. Neurotoxicology 1992; 13(3):637-48.

Reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, and hydroxyl radicals are believed to be initiators of peroxidative cell damage. The paper focuses on the use of 2',7'-dichlorofluorescein-diacetate (DCFH-DA) to quantitate cerebral ROS as an index for neurotoxicity. This technique employs an assay of dichlorofluorescein (DCF), the fluorescent product of dichlorofluorescein (DCFH). Data from studies using various free radical generating systems, several iron chelators and hydroxyl radical scavengers suggest that DCFH oxidation may result in several reactive intermediates. In a biological system (synaptosomes isolated from untreated rats) DCF fluorescence was stimulated by ascorbate or FeSO4, while deferoxamine inhibited the ascorbate/FeSO4-induced stimulation of DCF formation. Two organometals, methylmercury (MeHg) and trimethyltin (TMT), known to produce neurotoxicity were tested. In vitro exposure to MeHg (10-20 microM) increased the rate of formation of ROS while TMT (5-40 microM) had no effect. The results demonstrate that DCF fluorescence provides a good measure of overall ROS formation in synaptosomes of both in vitro as well as in vivo systems. Since ROS formation was selectively increased in areas known to be specifically vulnerable to organometals (cerebellum in the case of MeHg and hippocampus in the case of TMT), these studies further support that oxidative damage may be the primary mechanism underlying the neurotoxicity induced by these organometals.

85
Becking GC. METHODOLOGY IN NEUROTOXICOLOGY--ACTIVITIES WITHIN THE WORLD HEALTH ORGANIZATION AND INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY. Toxicol Lett 1992;64-65 Spec No:203-8.

For many years, the potential deleterious effects of

environmental factors on the human nervous system has been examined by several programmes within the World Health Organization (WHO). This presentation concentrates on the development of methodology to determine the health risks from chemical exposures both in the work place, as well as, the general environment. IPCS as a scientifically based cooperative programme of the United Nations Environment Programme, International Labour Organisation, and WHO, has as one of its goals the development and use of methods to assess human health and environmental risks from chemicals. In this report, emphasis was given to the development by IPCS of an integrated multidisciplinary approach for assessing the neurotoxic potential of chemicals and the risks to human health. The WHO neurobehavioural core battery developed within the Occupational Health Programme is described briefly.

OCULAR TOXICITY

86
Ikarashi Y, Tsuchiya T, Nakamura A. COMPARISON OF THREE IN VITRO ASSAYS TO DETERMINE THE OCULAR TOXICITY OF DETERGENT, OIL, AND ORGANIC SOLVENTS. J Toxicol Cutaneous Ocul Toxicol 1993;12(1):15-24.

The cytotoxicity of 39 chemicals, including detergents, oils, and organic solvents, previously reported in neutral red (NR) assay was further evaluated using Chinese hamster lung fibroblast V79 cells, primary rabbit corneal (RC) cells, and normal human epidermal keratinocytes (NHEK). Cationic detergents were most toxic in each cell type. Glycols and oils showed weak toxicity. NHEK cells showed higher sensitivity than other cell types. A good correlation was observed between in vitro cytotoxicity (IC50) and the eye irritation index (DS20 value) produced in the in vivo Draize test. Correlation coeffs. were V79 cells vs. DS20: r =0.93; RC cells vs. DS20: r = 0.92; and NHEK vs. DS20: r = 0.90. There was little difference in the predictability of eye irritancy of chemicals among three cell types. The NR cytotoxicity assay using V79 cells is useful for screening eye-irritating chemicals.

87
Li L, Hoffman RM. EYE TISSUES GROWN IN 3-DIMENSIONAL HISTOCULTURE FOR TOXICOLOGICAL STUDIES. J Cell Pharmacol 1992;2(6):311-16.

The authors report here the long-term three-dimensional

growth of human and mouse eye tissues, in particular conjunctiva and cornea, and their use in the development of an in vitro ocular safety assay. It was demonstrated that tissues of the eye can be grown as intact tissue with the maintenance of tissue architecture in a viable state on collagen-gel sponges in vitro for a relatively long period. Human normal conjunctiva can be histocultured for at least 7 days, and mouse conjunctiva can be histocultured for at least 30 days. To develop an in vitro ocular-safety assay, the fluorescent dyes BCECF-AM and propidium iodide (PI) were used to identify living and dead cells in the histocultured eye tissues with analysis of the three-dimensional cultures by confocal scanning laser microscopy. The end-point of incorporation of [3H]thymidine into the cells of the cultured cornea and conjunctiva as measured by histological autoradiography was also utilized. Ethanol was tested as a model toxin for the fluorescent-dye end point. The authors demonstrated an ethanol-toxicity dose response on histocultured human conjunctiva. To validate this methodology, ethanol toxicity on histocultured mouse eye tissue in vitro and the ethanol irritation of mouse eye tissue in vivo were compared and a high correlation was found. The long-term culture of conjunctiva that maintains intact tissue architecture such that in vitro toxicity correlates well with in vivo response should be useful for replacement of the controversial in vivo Draize test.

88
Martins T, Pauluhn J, Machemer L. ANALYSIS OF ALTERNATIVE METHODS FOR DETERMINING OCULAR IRRITATION. Food Chem Toxicol 1992;30(12):1061-7.

According to classification and labelling requirements, chemicals, dyes, agrochemicals and pharmaceutical formulations have to be evaluated for their potential to induce eye irritancy or corrosion. An attempt was made to analyse the predictive power of the bovine eye-chicken egg chorioallantoic membrane (BE-CAM) assay in comparison with results obtained using the conventional Draize method. In summary, results showed limited correlation between reactions in vitro and responses of eyes in vivo. In a pilot study, ultrasonic pachymetry showed high sensitivity and fairly good correlation between corneal thickness and clinical observations in eyes.

89
Matsuno H. BASIC STUDIES FOR ESTABLISHMENT OF ALTERNATIVES TO IN VIVO OCULAR TOXICITY TEST. TOXICITY OF LOCAL ANESTHETICS BY OPACITOMETER METHOD. Ou Daigaku Shigakushi 1992;19(1):28-46.

The objective of this study was to establish an in vitro opacitometer method as an alternative to the orthodox in vivo ocular irritancy testing (Draize method). Corneas isolated from the porcine eye balls which have been treated in vitro with various concentrations of lidocaine, procaine, prilocaine, and tetracaine were used in the in vitro opacitometer method. The in vivo Draize method was carried out for comparison using rabbit's eyes and 1 .times. 10-1M concentration of these local anesthetics. Then, in order to clarify the mechanism of these local anesthetics to produce porcine corneal opacification, using opacifying experimental egg white solution, corneal stroma (both the epithelium and endothelium-removed cornea), osmotic pressure measurement were carried out. There was a good correlation between % opacity of application to both surfaces of porcine intact cornea and score of Draize method. There was a good correlation between the

opacities developed when the local anesthetics were applied to the endothelial surface of intact cornea and the osmotic pressures measured. A lot of samples can be tested in a few hours. Ocular toxicity assessment by this method is objective and results are reproducible. Opacitometer method may have certain advantages over in vivo Draize method as well as over other alternative in vitro techniques.

90
Katsuta Y. STUDY ON THE ALTERNATIVE METHOD TO DRAIZE TEST. OCULAR IRRITANCY TOXICITY OF CHLOROPHENOLS BY OPACITOMETER METHOD. Ou Daigaku Shigakushi 1992; 19(1):47-73.

The Draize test has been criticized for the pain which animals are subjected to in this test. For these reasons, an alternative test method for ocular irritancy has been sought. The aim of this study was to evaluated the opacitometer method as a porcine ocular irritancy test in vitro. Three isomers of chlorophenol (o-, m- and p-chlorophenol) were used in three concentrations. Test compopunds were applied to the epithelial surface alone, the endothelial side

alone or both the epithelial and endothelial surfaces of the cornea. The degree of resultant corneal opacity was assessed with an opacitometer. Then, the Draize test was conducted to clarify the correlation between the data from the opacitometer method and the data from the Draize test. The results suggest that the major mechanism of chlorophenol induced corneal opacity is degeneration of the corneal epithelium. The opacitometer method is superior to the Draize test in the following aspects: it allows highly reproducible assessment, it is simpler and requires less time, it is cheaper (because a waste material is used), and it is not cruel to animals. Some unsolved problems of the opacitometer method are discussed.

91
Calvin G. NEW APPROACHES TO THE ASSESSMENT OF EYE AND SKIN IRRITATION. Toxicol Lett 1992;64-65(9):157-64. (41 REFS)

The present methodology for evaluation of the eye and skin irritation potential of chemicals and preparations new approaches to the assessment of eye and skin irritation (eye and skin irritation-in vivo and in vitro methods), and application in practice, and irritation assessment and regulation are discussed, followed by a brief summary and conclusions.

92
Rohde BH. IN VITRO METHODS IN OPHTHALMIC TOXICOLOGY. Ophthalmic Toxicol 1992;109-65. (88 REFS)

No abstract.

93
Decker D, Harper R. EVALUATION OF THE EYTEX SYSTEM FOR USE AS A PREDICTOR OF OCULAR IRRITANCY: I. SHAMPOOS. J Toxicol, Cutaneous Ocul Toxicol 1993;12(1):35-47.

The Eytex in vitro assay was used to evaluate 42 adult and baby shampoos. The assay, which is based on protein denaturation, was chosen because it provides rapid, quantitative results at a relatively low cost. All shampoos were diluted 1:10 in deionized water and tested with two different protocols: the rapid membrane assay (RMA) protocol for adult products and the high-sensitivity assay (HSA) for baby shampoos. All samples were tested in two and, in some cases, three

separate experiments and the qualified Eytex scores were averaged. The correlation of the Draize scores with the Eytex scores was statistically significant. The Eytex scores were used to establish irritation classes similar to the standard Draize classification scheme (minimal, mild, moderate, severe). When the Draize eye irritation class was compared to the Eytex irritation class for a given shampoo, the correlation for the baby shampoo data was 100% and 87% for the adult shampoo data. This study indicates that the Eytex in vitro assay for ocular irritancy can be highly predictive of Draize eye scores for shampoos. This suggests that such assays could be useful as screening tools in new shampoo development.

94
Chiou GC Y. TOXIC RESPONSES IN THE EYE AND VISUAL SYSTEM. Toxicol Methods 1992;2(3):139-67.

A review with 79 references on the anatomy and physiology of the eye, ophthalmic toxicol. research methods in vivo and in vitro, toxic responses induced by local agents and systemic drugs, and medical treatment of ocular toxic responses.

95
Ninomiya H, Kobayashi T. AN ALTERNATIVE METHOD OF DRAIZE TEST USING SWINE CORNEA IMPLANTED ON THE CHICK CHORIOALLANTOIC MEMBRANE. Fragrance J 1992; 20(8):55-60.

A review with 18 refereces on in vitro method for the determination of ocular irritancy using swine cornea implanted on the chick chorioallantoic membrane as an alternative to Draize test.

96
Sugai S, Murata K. INVESTIGATION OF THE IN VITRO EVALUATION METHOD OF EYE IRRITATION POTENTIAL USING RAT RED BLOOD CELLS. Fragrance J 1992;20(8):47-54.

A review with 22 references on investigation of the in vitro method for determination of eye irritation potential of chemicals using rabbit red blood cells as an alternative to the Draize test. The method involves the determination of the effects of chemicals on proteins and lipid membranes, evaluated by Hb denaturation and hemolysis, respectively. The in vitro

method applied to 117 chemicals including acids, bases, surfactants, and solvents correlated well with in vivo Draize eye irritation tests.

97
Bernstein PS, Lloyd MB, O'Day WT, Bok D. EFFECT OF PHYTANIC ACID ON CULTURED RETINAL PIGMENT EPITHELIUM: AN IN VITRO MODEL FOR REFSUM'S DISEASE. Exp Eye Res 1992;55(6):869-78.

Refsum's disease (heredopathia atactica polyneuritiformis) is an autosomal recessive retinitis pigmentosa syndrome caused by the excessive deposition of phytanic acid in ocular tissues. Efforts to elucidate the molecular mechanism of phytanic acid's retinal toxicity have been hampered by the rarity of human pathological specimens and by the inability to reproduce the disease in living animal models. In this study, an in vitro model for Refsum's disease was established by exposing cultured human and bovine retinal pigment epithelial cells to phytanic acid bound to bovine serum albumin at concentrations comparable to levels found in affected humans. Ultrastructural studies show that these cells exhibit morphological changes consistent with those observed in pathological specimens from patients with Refsum's disease. Biochemical assays of retinoid metabolism by cell membranes from control cells and from cells exposed to 200 microM phytanic acid demonstrate that the ability to esterify retinol and to isomerize ll-trans retinoids to 11-cis retinoids remains intact despite the deposition of large amounts of phytanic acid. The work described here is strong evidence against the hypothesis that phytanic acid inhibits vitamin A metabolism in the retinal pigment epithelium, and it demonstrates the potential use of cultured retinal pigment epithelial cells in modeling this and other degenerative diseases of the retina.

ORGAN CULTURE
98
Beele H, Thierens H, Deveux R, Goethals E, De Ridder L. SKIN ORGAN CULTURE MODEL TO TEST THE TOXICITY OF POLYOXYETHYLENE NETWORKS. Biomaterials 1992; 13(14):1031-1037.

Films of polyoxyethylene network were prepared from two types of triethoxysilane-terminated prepolymers. In this way, films of polyoxyethylene network with

possible applications in the biomedical field could be made easier. To test their biocompatibility, these networks were added to organ cultures of adult human skin and embryonic chicken skin. A rapid toxic effect was observed, especially with the urethane-linked network. Enzymatical degradation of the network by enzymes in the culture medium might be responsible for the formation of toxic metabolites. Testing of related chemical compounds in our in vitro assay suggested that the formation of a silane group with an amino terminal is most likely to be responsible for the toxic effects observed.

RESPIRATORY TOXICITY

99

Romet-Haddad S, Marano F, Blanquart C, Baeza-Squiban A. TRACHEAL EPITHELIUM IN CULTURE: A MODEL FOR TOXICITY TESTING OF INHALED MOLECULES. Cell Biol Toxicol 1992; 8(3):141-50.

Rabbit trachea primary cultures have been developed as a model to evaluate the toxicity of noxious airborne pollutants. A mucociliary epithelium has been restored in vitro on collagen gel. Several general cytotoxicity assays (viability and growth inhibition) permit a first assessment for the acute toxicity of the tested molecules. More specific criteria such as measurement of the integrity of the epithelial barrier and inhibition of ciliary beat frequency allow the determination of the specific impact of xenobiotics on the mucociliary epithelium in culture.

100

Wills-Karp M, Uchida Y, Lee JY, Jinot J, Hirata A, Hirata F. ORGAN CULTURE WITH PROINFLAMMATORY CYTOKINES REPRODUCES IMPAIRMENT OF THE BETA-ADRENOCEPTOR-MEDIATED RELAXATION IN TRACHEAS OF A GUINEA PIG ANTIGEN MODEL. Am J Respir Cell Mol Biol 1993;8(2):153-9.

The challenge of previously sensitized guinea pigs with aerosolized ovalbumin resulted in impairment of the beta- adrenoceptor-mediated relaxation as measured by the in vitro isometric assay of tracheas preconstricted with endothelin-1 or carbamylcholine. In order to investigate the pathophysiologic role of inflammation in hyperreactive airways, isolated guinea pig tracheas were cultured with proinflammatory cytokines such as human recombinant tumor necrosis factor-alpha

(TNF-alpha), interleukin-1 beta (IL-1 beta), or interleukin-2 (IL-2). None of these cytokines affected the contractile response of tracheas to carbamylcholine. After preconstriction with carbamylcholine, the TNF-alpha- and IL-1 beta-pretreated tissues produced a significant reduction in the maximal relaxation induced by isoproterenol, whereas the IL-2 pretreatment had no effect. The reduction of the isoproterenol-mediated relaxation by the IL-1 beta treatment was time and dose dependent. Present observations suggest that in vitro incubation of naive tracheas with proinflammatory cytokines is able to reproduce apparent beta-adrenoceptor impairment as seen in the airways of antigen-challenged guinea pigs of asthma model.

101
Ben-Jebria A, Marthan R, Savineau JP. EFFECT OF IN VITRO NITROGEN DIOXIDE EXPOSURE ON HUMAN BRONCHIAL SMOOTH MUSCLE RESPONSE. Am Rev Respir Dis 1992; 146(2):378-82.

The aim of the study was to develop an in vitro system in which an isolated bronchus from human lung was exposed, during 30 minutes, to a constant flow of either air or nitrogen dioxide (NO2), and to examine subsequently the contractile response of airway smooth muscle rings to carbachol, histamine, and substance P. Two proximal bronchi were mounted in an organ bath, perfused externally with Krebs-Henseleit solution and ventilated with clean air, 1.0 or 2.0 ppm NO2. The exposed bronchi were then cut into rings and mounted in a computerized organ bath system. Contractile responses to agonists were measured isometrically. In each ring, a cumulative concentration response curve was obtained to the desired agonist. In vitro exposure of the human lumen bronchus to 1.0 ppm NO2 did not significantly increase the efficacy or the potency of carbachol, exposure to 2.0 ppm NO2 increased airway smooth muscle contractions in response to carbachol, histamine, and substance P. The results indicate that the experimental preparation is well suited to study the respiratory toxicity of inhaled pollutants in order to understand further the mechanisms underlying toxicant-induced airway hyperresponsiveness.

102
Brain JD. SHORT-TERM IN VITRO AND IN VIVO BIOASSAYS: THEIR ROLE IN ESTIMATING THE TOXIC POTENTIAL OF INHALED

COMPLEX MIXTURES FOR HUMANS. Cell Biol Toxicol 1992; 8(3):123-32. (30 REFS)

No abstract.

103
Thoren SA. CALORIMETRY: A NEW QUANTITATIVE IN VITRO METHOD IN CELL TOXICOLOGY. A DOSE/EFFECT STUDY OF

ALVEOLAR MACROPHAGES EXPOSED TO PARTICLES. J Toxicol Environ Health 1992;36(4):307-318. (20 REFS)

A new short term test for measuring the effect of particles on alveolar macrophages was evaluated in cells from New-Zealand-white-rabbits and Wild-Colored-Dutch-rabbits. Alveolar macrophages were collected by bronchopulmonary lavage and were prepared as nonconfluent monolayers. Plates with adherent macrophages were placed in calorimetric ampules. Alveolar macrophages in monolayers were exposed to particles of quartz, titanium-dioxide, and manganese-dioxide. Heat flux values and heat flux time curves were determined for ampules containing exposed and control macrophages. The findings demonstrated that calorimetry can be used in toxicology successfully. The metabolic activity was measured as the heat exchange rate in the monolayers. The calorimeter detected toxic effects from quartz in the form of increased metabolic activity of exposed cells. This contrasted with the findings of the image analyzer. This supports the finding that silica particles can cause chronic modification of the macrophage function. The author suggests that this change in the alveolar macrophage function may be the first in a series of processes resulting in pulmonary fibrosis.

TERATOGENICITY
104
Toraason M, Bohrman JS, Krieg E, Combes RD, Willington SE, Zajac W, Langenbach R. EVALUATION OF THE V79 CELL METABOLIC CO-OPERATION ASSAY AS A SCREEN IN VITRO FOR DEVELOPMENTAL TOXICANTS. Toxicol In Vitro 1992;6(2). (40 REFS)

The utility of the V79 cell metabolic cooperation assay as an in-vitro screen for developmental toxicants was evaluated. Thirty-eight coded chemicals were tested for their effect on intercellular communication. A

separate cytotoxicity assay was used to determine the concentration of each chemical that inhibited clonal expansion of V79 cells. Only seven of the 29 designated teratogens proved positive for inhibition of intercellular communication. Four teratogens and one nonteratogen inhibited intercellular communication at only a single concentration or at cytotoxic concentrations and were considered equivocal. The sensitivity of the assay for teratogens was therefore in the range of 24 to 38%. The overall accuracy for correctly identifying teratogens and nonteratogens was in the range of 42 to 50%. Specificity ranged from 89 to 100%. The authors conclude that chemicals which inhibit intercellular communication under the given conditions have a high probability of being teratogens.

105
Turton JA, Willars GB, Haselden JN, Ward SJ, Steele CE, Hicks RM. COMPARATIVE TERATOGENICITY OF NINE RETINOIDS IN THE RAT. Int J Exp Pathol 1992;73(5):551-563.

The comparative teratogenicity of nine retinoids in Wistar rats was investigated. The compounds studied and dose levels tested (mg/kg) were: all-trans-retinoic acid (TRA), 6.25, 12.5, 25, 50, 100; etreteinate (eTR), 25, 50 acitretin (ACIT), 25, 50; 13-cis-retinoic acid (13CRA), 100, 200; and five retinamides, each at 300 and 600 mg/kg, N-(4-hydroxyphenyl)reteinamide (4HPR); N-tetrazol-5-ylretinamide (TZR); N-butylretinamide (NBR); N-ethylretinamide (NER); 13-cis-N-ethylretinamide (13CNER). Retinoids were administered by oral intubation on days 10 and 11 post coitum (p.c.). Dams were killed on day 22 p.c. and examinations carried out to assess teratogenic potential. Using a number of criteria a generalized ranking order of the toxicity of the compounds was drawn up: TRA > ETR > ACIT > 13CRA > 4HPR > TZR. Relative in-vivo teratogenicity for the nine retinoids is compared with a previously reported in-vitro assessment of the compounds using a rat whole embryo culture technique.

VALIDATION TESTS
106
Scott RC, Batten PL, Clowes HM, Jones BK, Ramsey JD. FURTHER VALIDATION OF AN IN VITRO METHOD TO REDUCE THE NEED FOR IN VIVO STUDIES FOR MEASURING THE ABSORPTION OF CHEMICALS THROUGH RAT SKIN. Fundam Appl Toxicol 1992;19(4):484-92.

Current requirements for the registration of agrochemicals, particularly in the U.S.A., often require the provision of dermal absorption data. In this process the rat is often used and complex in vivo studies, using large numbers of animals, are performed. We have compared the data obtained from in vivo and in vitro dermal absorption studies using eight pesticides with a range of physico-chemical properties. Measurements were made of the 14C-labeled pesticides which could be washed from the skin, were associated with (on/in) skin, or absorbed through the skin following dermal applications in vivo and in vitro at various time points over a 24-hr exposure period. Good agreement was found between the amounts washed from and associated with the skin in vivo and in vitro. The results indicate that, with a range of pesticide molecules, the in vitro method accurately predicted in vivo absorption supporting the utilization of the in vitro method for risk assessment from exposure to pesticides and other chemicals.

107
Li L, Hoffman RM. EYE TISSUES GROWN IN 3-DIMENSIONAL HISTOCULTURE FOR TOXICOLOGICAL STUDIES. J Cell Pharmacol 1992;2(6):311-16.

The authors reported the long-term three-dimensional growth of human and mouse eye tissues, in particular conjunctiva and cornea, and their use in the development of an in vitro ocular safety assay. It was demonstrated that tissues of the eye can be grown as intact tissue with the maintenance of tissue architecture in a viable state on collagen-gel sponges in vitro for a relatively long period. Human normal conjunctiva can be histocultured for at least 7 days, and mouse conjunctiva can be histocultured for at least 30 days. To validate the methodology, ethanol toxicity on histocultured mouse eye tissue in vitro and the ethanol irritation of mouse eye tissue in vivo were compared and a high correlation was found. The long-term culture of conjunctiva that maintains intact tissue architecture such that in vitro toxicity correlates well with in vivo response should be useful for replacement of the controversial in vivo Draize test.

108
Calvin G. NEW APPROACHES TO THE ASSESSMENT OF EYE AND

SKIN IRRITATION. Toxicol Lett 1992;64-65 Spec No:157-64. (14 REFS)

Assessment of eye and skin irritation potential is an important part of any comprehensive toxicology programme for new chemicals and consumer products. The original skin and eye irritation assessment methods described by Draize et al. are still widely used for regulatory purposes with only slight modification. These methods have been re-evaluated in the light of the modern approach to toxicological testing requiring refinement of protocols, reduction in animal numbers, and replacement of in vivo tests by in vitro assays. A refinement in the original Draize skin irritation test accepted by regulatory authorities is reduction of test substance exposure time from 24 to 4 h. This makes the test less stressful. The Low Volume Eye Irritation Test is also a less stressful, more predictive refinement of the Draize eye irritation procedure. Many in vitro models are being developed for use in the assessment of eye and skin irritation potential in the hope that the animal tests may some day be replaced. In order for alternative tests to be incorporated into safety assessment, they must undergo a defined evaluation that allows toxicologists to determine whether the new procedures provide relevant information. As the validity of the methods is determined, the new procedures can be incorporated into safety assessment using a tier assessment process. If alternative procedures are to be ultimately accepted for use in safety assessment, regulatory authorities must become active participants in programmes designed to assess and validate the new tests.

109
Hotchkiss S AM, Hewitt P, Caldwell J, Chen WL, Rowe RR. PERCUTANEOUS ABSORPTION OF NICOTINIC ACID, PHENOL, BENZOIC ACID AND TRICLOPYR BUTOXYETHYL ESTER THROUGH RAT AND HUMAN SKIN IN VITRO: FURTHER VALIDATION OF AN IN VITRO MODEL BY COMPARISON WITH IN VIVO DATA. Food Chem Toxicol 1992;30(10):891-899.

The in vivo percutaneous absorption of three model compounds, nicotinic acid, phenol and benzoic acid, and the herbicide triclopyr butoxyethyl ester (triclopyr BEE) has been investigated in flow-through diffusion cells using skin from male Fischer 344 rats and humans. After the application of four chemicals to the epidermal surface of unoccluded full-thickness rat skin, the absorption of each compound across the skin

and into the receptor fluid at 72 hr reached 3.7 : 0.3, 5.7 : 0.6, 26.7 : 3.7 a phenol and benzoic acid, respectively. After the application of the four chemicals to the epidermal surface of unoccluded full-thickness human skin, the absorption of each compound across the skin and into the receptor fluid at 72 hr was significantly ($P < 0.05$) less than through rat skin, reaching 0.7 : 0.1, 0.7 : 0.2, 18.8 : 1.3 and 37.8 : 6.9% (mean : SD, n = 2-7) of the applied dose for triclopyr BEE, nicotinic acid, phenol and benzoic acid, respectively. The in vitro data reported compare favourably with data obtained by other workers using both in vitro and in vivo methodologies. The in vitro:in vivo correlation supports the use of the flow-through diffusion cell system as a model for the prediction of percutaneous absorption in vivo in the rat and in humans.

110
Harvell J, Bason MM, Maibach HI. IN VITRO SKIN IRRITATION ASSAYS: RELEVANCE TO HUMAN SKIN. J Toxicol Clin Toxicol 1992;30(3):359-369.

Animals have been used to assess dermal irritation by observation of visible changes ranging from erythema and edema to corrosion and ulceration in the in vivo Draize rabbit skin test accepted by many regulatory agencies. These responses easily observed, are produced by diverse mechanisms. Investigators have found that different species exhibit widely varying reactivity under identical test conditions especially using substances with only minor irritant potential. Thus, the accuracy of the Draize test and other animal testing as it relates to humans has been called into question. Also, the results from the animal methods currently used differ due to the subjective visual test scoring. These differences occur most frequently in the assessment of the toxicity of mild irritants and colored material. Thus, it has been postulated that animals should not be the exclusive means of evaluation. Human considerations have galvanized efforts to find alternative testing methods. The development of an in vitro irritation assay may be a means of improving upon the accurracy of the animal test while reducing the number of animals needed to test a given compound. In vitro skin irritation test are being developed in the hope that methods of analysis can be determined that are more humane, more predictive of actual human response, and will provide an objective quantifiable means of determining the

irritancy potential of a substance - a major advantage compared to subjectively assessed animal tests. The review attempts to summarize previous studies, and proposes a strategy of validating such assays.

111
Scott RC, Batten PL, Clowes HM, Jones BK, Ramsey JD. FURTHER VALIDATION OF AN IN VITRO METHOD TO REDUCE THE NEED FOR IN VIVO STUDIES FOR MEASURING THE ABSORPTION OF CHEMICALS THROUGH RAT SKIN. Fundam Appl Toxicol 1992;19(4):484-92.

Current requirements for the registration of agrochemicals, particularly in the U.S.A., often require the provision of dermal absorption data. In this process, the rat is often used and complex in vivo studies, using large numbers of animals, are performed. The authors compared the data obtained from in vivo and in vitro dermal absorption studies using eight pesticides with a range of physicochemical properties. Measurements were made of the 14C-labeled pesticides which could be washed from the skin, were associated with (on/in) skin, or absorbed through the skin following dermal applications in vivo and in vitro at various time points over a 24-h exposure period. Good agreement was found between the amounts washed from and associated with the skin in vivo and in vitro. Over the time period 4-24 h after application, the in vitro experiments predicted the in vivo absorption within a factor of 2-3. These results show that, with a range of pesticide molecules, the in vitro method accurately predicted in vivo absorption supporting the utilization of the in vitro method for risk assessment from exposure to pesticides and other chemicals.

112
Mayr U, Butsch A, Schnieder S. VALIDATION OF TWO IN VITRO TEST SYSTEMS FOR ESTROGENIC ACTIVITIES WITH ZEARALENONE, PHYTOESTROGENS AND CEREAL EXTRACTS.

Toxicol 1992;74(2-3):135-149.

In order to establish alternatives to the frequently used uterotropic assay with mice, defined estrogen-sensitive cell lines (MCF-7 cells and LeC-9 cells) were used to determine the estrogenic activities of purified compounds of vegetable origin (myco- and phytoestrogens) and zearalenone-contaminated forage cereals (wheat, barley and oats). In MCF-7 cells, a

human breast cancer cell line, the induction of an estrogen-specific exoprotein served as a parameter of estrogenic activities. LeC-9 cells represent a genetically transformed cell clone derived from mouse L-cells. Here, hormone-like activities were measured by the expression of the bacterial chloramphenicol acetyltransferase (CAT) gene under the control of an estrogen-responsive element. Toxic effects affecting cell viability were monitored in this system by the expression of a second reporter gene (the bacterial beta-galactosidase gene controlled by the constitutive human beta-actin promoter). Relative estrogenic activities of myo- and phytoestrogens determined with both systems are concomitant, but greater as compared to the uterotropic assay with mice.

113
Jellett JF, Marks LJ, Stewart JE, Dorey ML, Watson-Wright W, Lawrence JF. PARALYTIC SHELLFISH POISON (SAXITOXIN FAMILY) BIOASSAYS: AUTOMATED ENDPOINT DETERMINATION AND STANDARDIZATION OF THE IN VITRO TISSUE CULTURE BIOASSAY, AND COMPARISON WITH THE STANDARD MOUSE BIOASSAY. Toxicon 1992;30(10):1143-56.

Mouse neuroblastoma cells swell and eventually lyse upon exposure to veratridine, which, when added together with ouabain, enhances sodium ion influx. In the presence of saxitoxin (STX), which blocks sodium channels, the action of the other two compounds is inhibited and the cells remain morphologically normal. A tissue culture bioassay using mouse neuroblastoma cells, developed by Kogure and colleagues, takes advantage of these principles; in this bioassay, the fraction of the cells protected from the actions of ouabain and veratridine is in direct proportion to the concentration of STX and its analogues. The authors have modified this bioassay, improving its convenience and speed by eliminating the need to count individual cells to determine the saxitoxin equivalents, and instead have employed a microplate reader for automated determinations of absorbances of crystal violet from stained neuroblastoma cells. The automated tissue culture (neuroblastoma cell) bioassay may be a valid alternative to live animal testing for paralytic shellfish poisoning.

114
Li Q, Aoyama K. STUDY OF DOSE-RESPONSE RELATIONSHIP IN CONTACT SENSITIVITY USING AN IN VITRO ASSAY. Contact

Dermatitis 1992;27(1):16-21.

Dose-response relationships in contact sensitivity were evaluated in guinea pigs using an in vitro assay. Guinea pigs were sensitized with different doses of 1-chloro-2,4-dinitrobenzene (DNCB) and challenged with DNCB and 2,4-dinitrobenzene sulfonic salt (DNBS). Lymph node cells from sensitized and control guinea pigs were cultured in the presence of different doses of DNCB and DNBS at 8 x 10(5) cells/well, respectively. The sensitivity was evaluated by the lymphocyte transformation test (LTT), which was assessed by uptake of 3H-thymidine. The results indicated that there were significant correlations between the doses of sensitizers and the values of LTT in both phases of induction and challenge. Thus, the presence of higher numbers of LTT-reactive lymphocytes in the circulation may well correlate with the sensitizing doses. The in vitro LTT may discriminate between positive patch test reactions and negative or doubtful reactions, but not between weak positive and strong positive reactions. The in vitro assay reproduced the cross-reaction between DNCB and DNBS which was confirmed in vivo.

VASCULAR TOXICITY

115

Borgs P, Way DL, Witte MH, Case TC, Ramirez GJ, Witte CL. EVALUATING IN VITRO TOXICITY TO MAMMALIAN ENDOTHELIAL CELLS. In Vitro Methods Toxicol 1992;269-84.

A review with 56 refs. on the central role of the endothelial cell, available endothelial cell culture systems, methods to delineate toxicity in vitro, and basic toxicological principles applicable to mammalian endothelial cell culture systems. The authors then comment on the limitations and potential value of these in vitro models in providing insight into vascular toxicity in vivo.

XYZ/MISCELLANEOUS

116

Young RJ, Bodt BA, Iturralde TG, Starke WC. AUTOMATED ANALYSIS OF RABBIT SPERM MOTILITY AND THE EFFECT OF CHEMICALS ON SPERM MOTION PARAMETERS. Mol Reprod Dev 1992;33(3):347-56.

Appropriate software settings and optimum procedures were determined for the measurement of the motion parameters of rabbit spermatozoa by the CellSoft (Cryo Resources Ltd., Montgomery, NY) computer-assisted

digital image analysis system. The system was used to follow motion parameter changes occurring in spermatozoa incubated for 6 h with or without exposure to chemicals. The initial decrease in Vc was dependent on the concentration of the added compound. Motion-based indexes-motility concentration (MCl50), motility time (MTI50) and velocity (VI)-were defined and used as toxicology endpoints. The rank order of these indexes, the end point of the neutral red in vitro assay for cytotoxicity, and LD50 values for the five compounds were the same, suggesting that chemical inhibition of sperm motility may be useful as a method for the in vitro assessment of chemical cytotoxicity.

117
Darroudi F, Farooqi Z, Benova D, Natarajan AT. THE MOUSE SPLENOCYTE ASSAY, AN IN VIVO/IN VITRO SYSTEM FOR BIOLOGICAL MONITORING: STUDIES WITH X-RAYS, FISSION NEUTRONS AND BLEOMYCIN. Mutat Res 1992;272(3):237-48.

A modified mouse splenocyte culture system was standardized after testing different mitogens (i.e., phytohemagglutinin (PHA), concanavalin A (Con A)). The mitotic index was determined for comparison between different mitogens. Following selection of appropriate mitogen (PHA 16, Flow), a series of experiments were conducted to evaluate the application of a cytokinesis-block for scoring micronuclei and assays for chromosomal aberrations produced by treatment in G0 and G2 for the purposes of biological dosimetry following in vivo and/or in vitro exposure to X-rays, fission neutrons and bleomycin.

118
Le Goff L, Lapeyrade D, Bossi A, Noel-Hudson MS, Bonaly J, Wepierre J. EFFECT OF BIOPHYSICAL CHANGES ON PROPIDIUM IODIDE ACCESS TO DNA DURING OXIDATIVE STRESS OF CULTURED HUMAN SKIN CELLS. Toxicol In Vitro 1992; 6(5):423-432.

The sensitive single-cell analytical techniques of flow cytometry and propidium iodide-binding have been used to examine the molecular effect of oxidative stress on cultured human skin fibroblasts. Cells synchronized by limited time attachment were exposed to a hypoxanthine-xanthine oxidase (HX/XO) system at different intervals after subculture. The characteristic feature of the treated population was a variation of the amount of nuclear DNA propidium iodide

(PI)-fluorescence staining. Increased fluorescence intensity was observed with a shift of the G1/G0 and G2/M peak, which is dependent on both cell cycle stage and treatment level. When scavenger molecules (catalase, silybin) were added to the oxidative reaction, the nuclear DNA histogram of HX/XO-treated cells was similar to that obtained from untreated cells. In parallel, UV absorbance studies in vitro have shown that PI is capable of binding extensively to DNA when isolated from HX/XO-exposed cells, compared with control cells or HX/XO-exposed cells in the presence of scavengers. These results indicate that free radicals are responsible for the increase in fluorescence intensity in the HX/XO-exposed cells. This change is DNA stainability would be due to an opening of the DNA strands in situ, leading to an unmasking of new PI-binding sites on DNA. The strand separation may facilitate access, on the fluorochrome to DNA, thereby enhancing dye binding. This flow cytometric assay based on DNA biophysical changes caused by free radicals is a useful means of measuring pro- and/or anti-oxidant potential.

119
Rees RW, Amphlett NW, Chambers GA, De Mitchell I. METHOD DEVELOPMENT FOR THE IN-VIVO-IN-VITRO RAT HEPATOCYTE UNSCHEDULED DNA SYNTHESIS ASSAY AND A PROPOSAL FOR MULTIPLE DOSING WITH SINGLE HARVESTING TIME. Joint Meeting of the United Kingdom Environmental Mutagen Society/DNA Repair Network on Single Cell Gel Electrophoresis, Wales, England, UK, March 23-27, 1992. Mutagenesis 1992;7(5):384.

No abstract.

120
Wiegand-Rosinus M, Obst U, Haberer K, Wild A. ENZYMES IN VITRO AS INDICATORS FOR PESTICIDES: AN EXAMINATION. Environ Toxicol Water Qual 1992;7(4):313-321.

Pesticides-a serious problem especially for drinking water quality- frequently are potent inhibitors of enzymes in their target organisms. As the established chemical analyses of pesticides are time-consuming, complicated, and expensive, so-called screening methods are urgently needed. For this purpose the sensitivity of 13 different enzymes was tested in vitro by inhibiting their kinetic rates and/or substrate conversion by 16 pesticides (herbicides and

fungicides). It was shown that the sensitivity to pesticides of the different enzymes varies in a wide range, partly in irrelevant high concentrations. Four enzymes seem to be suitable for inhibition tests, but need to be checked before practical application. The enzyme aldehyde dehydrogenase (AIDh), isolated from yeasts, was very sensitive to ethendithiocarbamates. Therefore an inhibition test could be developed to detect this class of pesticides in a range of some mug/L. The AIDh inhibition test is the first part of an enzymatic screening system for pesticide pollution in water.

121
Abbott BD, Diliberto JJ, Birnbaum LS. MECHANISMS OF TCDD-INDUCTION OF CLEFT PALATE INSIGHTS FROM IN-VIVO AND IN-VITRO APPROACHES. Eleventh International Symposium On Chlorinated Dioxins And Related Compounds 1991, Part I, Research Triangle Park, North Carolina, USA, September 23-27, 1991. Chemosphere 1992; 25(1-2):75-78.

No abstract.

122

Anon. DRUG AND PHARMACEUTICAL TOXICITY: ALTERNATIVES TO ANIMAL TESTING. (Latest citations from the Life Sciences Collection Database). National Technical Information Service, Springfield, VA, USA. Gov Rep Announce & Index (GRA&I), Issue 24, 1992.

The bibliography contains citations concerning in vitro toxicity testing of drugs and pharmaceuticals. Test methods focus on genetic damage assessment and include the Ames test, sister chromatid exchange, SOS chromotext, UMU and REC assays. Effectiveness in determining the toxicity of antineoplastic drugs is reviewed in great detail. The citations also explore the toxicity of drugs such as antibacterials, xenobiotics, psychotropics, analgesics, anticonvulsants, antihypertensives, anti-inflammatories, and fertility enhancers. (Contains 250 citations and includes a subject term index and title list.) Published Search. Prepared in cooperation with Cambridge Scientific Abstracts, Washington, DC. Sponsored in part by National Technical Information Service, Springfield, VA.

123
Gettings SD. UPDATE ON IN-VITRO ALTERNATIVES DEVELOPMENT. Cosmet Toilet 1992;107(10):71-83. (73 REFS)

A review of the Cosmetic, Toiletry and Fragrance Association's Evaluation of Alternatives Program, which describes in vitro assays as alternatives to animal testing for dermal and eye irritation testing, is presented; examples of test materials, specific in vitro tests, and strengths and weaknesses of current tests are included.

124
Williams CD, Faisal M, Huggett RJ. POLYNUCLEAR AROMATIC HYDROCARBONS AND FISH LENS CATARACT EFFECTS OF BENZO-A-PYRENE-7 8-DIHYDRODIOL ON THE MACROMOLECULAR SYNTHESIS OF CULTURED EYE CELLS. Sixth International Symposium on Responses of Marine Organisms to Pollutants, Woods Hole, Massachusetts, USA, April 24-26, 1991. Mar Environ Res 1992;34(1-4):333-337.

No abstract.