

Alternatives to the Use of Live Vertebrates in Biomedical Research and Testing: Second Annual Annotated Bibliography

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Introduction

Because of considerable interest from Congress, the National Institutes of Health (NIH), and the public about animal welfare and alternatives to animal testing, the National Library of Medicine (NLM) searches its online databases and prepares quarterly annotated bibliographies on alternative or in vitro methods for toxicity testing and biomedical research. The objective is to present current literature organized as citations with brief annotations for easy scanning.

The Institute of Laboratory Animal Resources (ILAR) has invited NLM to publish *in ILAR News* this annual supplement, which is an edited, concatenated version of the quarterly bibliographies. *The ILAR News* Editorial Panel and outside reviewers edit and condense the quarterly bibliographies so the entries are appropriate for *the ILAR News* audience.

The scientific community is concerned about humane animal care and is sensitive to public concerns about how and why animals are used in biomedical research and toxicity testing. The following events reflect the involvement of the public and the U.S. government in this issue: an array of federal legislation related to animal welfare and the use of laboratory animals, U.S. Public Health Service policy on the humane care and use of laboratory animals, and efforts at NIH to promote and support a search for alternative methods to the use of animals in biomedical research and testing.

Scientists generally view the use of laboratory animals in biomedical research and toxicity testing as necessary except where valid scientific alternative methods will satisfy all testing requirements. However, when animals must be used, the scientific community supports careful consideration of the number of animals used and encourages reductions when they are scientifically feasible.

Therefore, by providing the scientific community with access to its bibliographic databases and by producing bibliographies on animal alternatives, NLM is supporting efforts by the NIH to increase the knowledge needed to develop methods of biomedical research and experimentation that

- do not require the use of vertebrate animals,
- reduce the number of vertebrate animals used in research,
- produce less pain and distress in vertebrate animals than do current methods,
- validate or demonstrate the reliability of nonanimal methods, and
- increase the use of nonvertebrate animal research methods that have been found to be valid and reliable.

The NLM anticipates an acceleration in the development of alternative or in vitro methods used in toxicity testing and biomedical experimentation, which will result in more articles about these methods in the literature.

Carcinogenicity

Ritzi, E. M., and S. Pyo. 1989. Levels of viral glycoprotein provide a dose-dependent measure of tumor cell inhibition. *Life Sci.* 44(26):2041-2050.

A chemosensitivity assay using small replicate Mm5mt/cl C3H mammary tumor cell cultures was developed to determine whether changes in viral antigen expression and release into culture fluids could be used as an in vitro indicator of chemotherapeutic drug effect. The 52,000 molecular weight viral envelope glycoprotein of the mouse mammary tumor virus was measured in culture medium of control and drug-treated cultures while cell density was simultaneously determined by cell staining and absorption at 664 nm. The dual measures of therapeutic effect afforded by this assay support its use as an in vitro measure for optimizing drug treatments.

Steele, V. E., J. T. Arnold, J. Van Arnold, and M. J. Mass. 1989. Evaluation of a rat tracheal epithelial cell culture assay system to identify respiratory carcinogens. *Environ. Mol. Mutagen.* 14(1):48-54.

To evaluate a short-term epithelial cell assay system for detecting respiratory carcinogens, primary cultures of rat tracheal epithelial cells were exposed to a series of 17 compounds and scored for morphologically transformed cell colonies 28 days later. Results indicate that the assay may be useful in identifying potential respiratory carcinogens in the environment.

Cell Transformation

Birek, C., T. Pawson, C. A. McCulloch, and H. C. Tenenbaum. 1988. Neoplastic transformation of osteogenic cells: Quantitative morphometric analysis of an in vitro model for osteosarcoma. *Carcinogenesis* 9(10): 1785-1791. A series of histopathologic parameters of neoplastic transformation and osteogenesis were quantitated, at a single cell level, by computer-assisted morphometry. Taken together, the data show that in this in vitro system, neoplastic transformation of osteogenic cells does occur, changes in osteoid and bone production are related to neoplastic transformation, and osteosarcoma-like changes can be quantitated at the individual cell level

Commentary

Allison, R. 1989. The animal research issue [Letter]. *J. Nat. Cancer Inst.* 81(17): 1332-1334.

Cheong, J. 1989. The use of animals in medical education: A question of necessity vs. desirability. *Theor. Med.* 10(1):53-57.

An issue in current animal welfare ethics is the use of animals in medical education. At stake is the conflict of pain and suffering of the animals versus the benefit to the students. The educator's role is to balance these two concepts. If the animals do suffer, this has to be justified by clearly establishing the necessity for their use. This discussion addresses the arguments for and against animal use, alternatives, and proposals for the resolution of the controversy.

English, D. C. 1989. Using animals for the training of physicians and surgeons. *Theor. Med.* 10(1):43-52.

The case is made that alternatives to animals can replace animal use in much of current medical education. Medical educators should routinely question and offer adequate justification for any use of animals in medical education. An assessment of one course used for ob-gyn training is presented.

Flint, O. P. 1988. In vitro toxicology: A commercial proposition? *Xenobiotica* 18(6):707-714.

When the in vitro test is a satisfactory predictive model of toxicity there are sound commercial reasons for its use. It saves time and effort and can be used at an early stage in compound development to sort out the toxic from the nontoxic candidate compounds. Large numbers of structurally similar compounds can be tested (QSAR studies), and an understanding of the mechanism of toxic action is often greater than from in vivo tests because of the numbers of animals required and the interplay of different organ systems in the toxic events that make interpretation difficult. Thus the in vitro test not only saves animals but also saves compounds.

Goldberg, A. M., and J. M. Frazier. 1989. Alternatives to animals in toxicity testing. *Sci. Am.* 261(2):24-30 (5 refs.).

Green, M. H. 1988. Short-term tests and the myth of the nonclastogenic mutagen. *Mutagenesis* 3(5):369-371 (7 refs.).

There are several good reasons for retaining mammalian cell mutation assays in a basic test package for genotoxicity and that the possible existence of a nonclastogenic mammalian mutagen is not one of them. A number of propositions concerning in vitro assays are presented, including their need for repetition, the inappropriateness of rodent carcinogenicity as a reference point for validation, the need for assays to corroborate rather than complement each other, the need for ongoing validation and development, and the need for as complete an in vitro package as possible in order to avoid unnecessary animal experimentation.

Rauhut, W. 1988. [Necessary data with regard to laboratory animals and in vivo experiments]. *Wiad. Parazytol.* 34(4-6):683-686.

Cytogenetics

Degrassi, E. and C. Tanzarella. 1988. Immunofluorescent staining of kinetochores in micronuclei: A new assay for the detection of aneuploidy. *Mutat. Res.* 203(5):339-345.

The immunofluorescent staining of kinetochores in micronuclei with antikinetochores antibodies was used to develop an in vitro assay for aneuploidy-inducing agents. Results indicate that the in vitro micronucleus assay coupled with immunofluorescent staining of kinetochores can be a useful method for assessing the ability of chemicals to induce aneuploidy, chromosome aberrations, or both.

Dulout, F. N., and A. T. Natarajan. 1987. A simple and reliable in vitro test system for the analysis of induced aneuploidy as well as other cytogenetic end-points using Chinese hamster cells. *Mutagenesis* 2(2): 121-126. A procedure is described for induction of aneuploidy and for the screening of aneuploidy-inducing agents using primary cell cultures of Chinese hamster embryo cells grown on cover glasses. To avoid the excessive scattering and subsequent loss of chromosomes, a hypo-tonic treatment with a 0.17 percent sodium chloride solution, at room temperature, followed by in situ fixation was standardized. This procedure improved the method through the reduction of the spontaneous frequency of aneuploid cells. Accuracy of the system was demonstrated. The average chromosome number remained constant in spite of the induction of high frequencies of aneuploid cells. Moreover, the method allows for the analysis of other cytogenetic end points such as anaphase-telophase alterations, structural chromosome aberrations, or sister chromatid exchanges.

Lindahl-Kiessling, K., I. Karlberg, and A. M. Olofsson. 1989. Induction of sister-chromatid exchanges by direct and indirect mutagens in human lymphocytes, co-cultured with intact rat liver cells. Effect of enzyme induction and preservation of the liver cells by freezing in liquid nitrogen. *Mutat. Res.* 211(1):77-87.

An in vitro assay system was developed that uses intact rat hepatocytes and human peripheral lymphocytes with the aim of bringing test conditions closer to in vivo conditions and thereby broadening the available battery of simple in vitro assays. Determination of sister-chromatid exchange rate was the experimental end point. The assay system was validated using a series of previously tested chemicals. It was further shown that the metabolic capacity of both normal and induced liver cells can be preserved in liquid nitrogen for long periods.

Maier, P. 1988. Development of in vitro toxicity tests with cultures of freshly isolated rat hepatocytes. *Experientia* 44(10):807-817.

Drug-induced pathological alterations in ploidy in hepatocyte cultures can serve as indicators of compounds, such as liver tumor promoters, which interfere with cell differentiation in liver. Coculturing freshly isolated hepatocytes with isolated, in vitro-cultured, rat liver epithelial cells can considerably increase the amount of information available from in vitro studies. Combined monitoring of cellular DNA (ploidy) and protein content in hepatocyte cultures during and after exposure to a given test compound at tissue oxygen tension with the heterotypic cell-cell interaction will create a more in vivo-like culture system. This will enhance the predictability of hepatocyte cultures and contribute to a more widespread use of the test system and as a result help to reduce the number of whole-animal tests.

Schmuck, G., G. Lieb, D. Wild, D. Schiffmann, and D. Henschler. 1988. Characterization of an in vitro micronucleus assay with Syrian hamster embryo fibroblasts. *Mutat. Res.* 203(6):397-404.

Using Syrian hamster embryo cells for assessing genotoxicity provides an opportunity to determine five different end points (gene mutations, DNA strand breaks, aneuploidy, DNA repair [unscheduled DNA synthesis, U-DS], and neoplastic transformation) in the one-cell system. This report concerns the characterization of an additional end point in the same cell system: the formation of micronuclei that indicate chromosomal changes induced by chemicals. Correlations between the formation of micronuclei and the Ames test, induction of UDS, cell transformation, and the in vivo bone marrow micronucleus test are demonstrated.

Sinha, A. K., B. B. Gollapudi, V. A. Linscombe, and M. L. McClintock. 1989. Utilization of rat lymphocytes for the in vitro chromosomal aberration assay. *Mutagenesis* 4(2): 147-153.

Cultured rat lymphocytes were used to evaluate an alternative cell system for in vitro cytogenetic assays. Unlike samples of human blood, rat blood can be collected under well-controlled environmental conditions. Because of the easy access to rat blood samples, the simplicity of culture, the reproducible nature of its in vitro growth, the positive response to known clastogens and negative response to media pH changes or hyperosmolarities, the rat lymphocyte in vitro chromosomal assay presented is an optimal system to assess the mutagenic potential of chemicals.

Wallin, M., B. Friden, and M. Billger. 1988. Studies of the interaction of chemicals with microtubule assembly in vitro can be used as an assay for detection of cytotoxic chemicals and possible inducers of aneuploidy. *Mutat. Res.* 201(2):303-311 (48 refs.).

The isolation and purification of microtubules and the effects of cytotoxic chemicals on microtubule assembly in vitro are reviewed. Because the cell mitotic spindle consists predominantly of microtubules, it is suggested that compounds inhibiting assembly might be potential inducers of aneuploidy.

Cytology

Bavister, B. D., and J. C. Andrews. 1988. A rapid sperm motility bioassay procedure for quality-control testing of water and culture media. *J. In Vitro Fert. Embryo Transf.* 5(2):67-75.

A rapid bioassay procedure is described for quality-control testing of water and apparatus used in the preparation of media for gamete and embryo culture. This bioassay is based on the sensitivity of hamster epididymal spermatozoa to contaminants present in water, the culture apparatus, or both. The bioassay is simple to perform and can be completed in one working day. It may be a useful alternative to the conventional mouse embryo tests that are widely used in human in vitro fertilization laboratories.

Carmichael, J., J. B. Mitchell, W. G. DeGraff, J. Gainson, A. F. Gazdar, B. E. Johnson, E. Glatstein, and J. D. Minna. 1988. Chemosensitivity testing of human lung cancer cell lines using the MTT assay. *Br. J. Cancer* 57(6):540-547.

Thirty human lung cancer cell lines were tested for chemosensitivity using the semiautomated, nonclonogenic MTT (based on reduction of a tetrazolium salt assay). This panel of lung cancer cells exhibited a drug sensitivity profile paralleling that observed in clinical practice. Results suggest that this lung cancer cell line panel in combination with a relatively simple but reproducible chemosensitivity assay, such as the MTT assay, has potential for testing drug combinations and evaluating new anticancer agents in vitro.

Dartsch, P. C., and E. Betz. 1989. Response of cultured endothelial cells to mechanical stimulation. *Basic Res. Cardiol.* 84(3):268-281.

An apparatus was constructed to imitate the volume pulse with its typical incisura of the abdominal aorta. Using this apparatus, cultured endothelial cells were exposed to continuously produced cyclic and directional stretching and relaxation for three days. In all experiments, cells remained attached and viable when subjected to mechanical stimulation. Results indicate that endothelial cell elongation and orientation in vitro can be induced by periodic stretching and relaxation comparable to the periodic oscillations of the vessel wall due to blood pulsation in vivo.

Korpela, M., and H. Tahti. 1988. The effect of in vitro and in vivo toluene exposure on rat erythrocyte and synaptosome membrane integral enzymes. *Pharmacol. Toxicol.* 63(1):30-32 (17 refs.).

The effects were compared of in vitro and in vivo exposure to toluene on erythrocyte and synaptosome membrane-bound acetylcholinesterase, total adenosine-triphosphatase, and magnesium-activated ATPase. The authors conclude that in vitro experiments can be used to predict in vivo toxic nerve cell membrane effects of organic solvents, and erythrocyte membrane is a suitable nerve cell membrane model for studies on anesthetic mechanisms of solvents.

Malorni, W., G. Formisano, and G. Donelii. 1989. Morphologic changes induced in vitro by 2,5 hexanedione. *In Vitro Cell. Dev. Biol.* 25(1):82-90.

The effects of 2,5 hexanedione (2,5 HD) were investigated in a human mammary carcinoma cell line, a human melanoma cell line, and fetal mouse neuronal cells in primary culture. Light and electron microscopic observations demonstrated that changes in cell proliferation can be detected. Furthermore, a noticeable increase in cell

protrusions and dendritic-like processes can occur. Differences in the features of these processes were detected between the different cell lines. These data can indicate nonneuronal cells as possible further targets of the toxicant. Results suggest that the sensitivity of in vitro systems may represent a useful tool for studying the mechanisms of action of the neurotoxicant 2,5 I-ID.

Oshiro, Y., R S. Balwierz, and C. E. Piper. 1988. Evaluation of the division arrest method of the CHO/HGPRT mutation assay. *J. Appl. Toxicol.* 8(2): 129-134.

The division arrest method of the CHO/HGPRT mutation assay following the procedure described by O'Neill et al. (*Environ. Mutagen.* 4:421-434, 1982) was evaluated. This method simplifies the culture manipulations required during the expression period and can be readily adopted for screening mutagenic compounds. Results indicate that the division arrest method of the CHO/HGPRT mutation assay can be reliably used for routine screening.

Rezabek, M. S., J. E. Trosko, C. Jone, and S. D. Sleight. 1988. Effects of hepatic tumor promoters phenobarbital and polybrominated biphenyls on intercellular communication between rat liver epithelial cells. *In Vitro Toxicol.* 2(1): 45-58 (74 refs.).

Intercellular communication assays were used to study the effects of FireMaster-BP-6, phenobarbital, and retinyl-acetate using a rat liver epithelial cell line. The authors conclude that although intercellular communication assays are potentially useful indicators for tumor-promoting compounds, caution must be used in interpreting the results because differences in cells, culture conditions, laboratory techniques, and type of assay may affect results.

Van Bossuyt, H., and E. Wisse. 1988. Culture Kupffer cells, isolated from human and rat liver biopsies, ingest endotoxin. *J. Hepatol. (Amsterdam)* 7(1):45-56.

A technique is described for the isolation and culture of Kupffer cells (Kc) from needle and surgical liver biopsies of humans and rats. Results suggest that the isolation and culture technique described seems to be a promising method for the in vitro study of human or rodent Kc when total intact livers are not available.

Cytotoxicity

Abe, M., I. Morita, and S. Murota. 1988. A new in vitro method using fura-2 for the quantification of endothelial cell injury. *Prostaglandins Leukotrienes Essent. Fatty Acids* 34(1):69-74.

Endothelial cell injury was determined by a new method based on measuring a specific release of fura-2, a fluorescent calcium indicator. We examined the cytotoxicity of 15-hydroperoxyeicosatetraenoic acid (15-HPETE) on cultured endothelial cells using the fura-2 assay. The fura-2 assay was so sensitive to the endothelial cell injury that the damage of the cells due to 15-HPETE at a dose as low as 5 /ml could be detected in as soon as 30 minutes. The new method may be useful for the approach to searching injurious substances or their antagonists to several kinds of cells.

Babich, H., and E. Borenfreund. 1987. Fathead minnow FHM cells for use in in vitro cytotoxicity assays of aquatic pollutants. *Ecotoxicol. Environ. Safety* 14(1):78-87.

The suitability of the fathead minnow (FHM) epithelial cell line for use as the target (indicator) system in in vitro cytotoxicity assays was evaluated using several end points. The neutral red assay was used to compare the relative sensitivities of the FHM cells with those of bluegill sunfish (BF-2) cells, a fibroblastic cell culture in the presence of different classes of test agents frequently occurring as aquatic pollutants. Overall, the FHM cells were more sensitive than were the BF-2 cells.

Babich, H., M. K. Sardana, and E. Borenfreund. 1988. Acute cytotoxicities of polynuclear aromatic hydrocarbons determined in vitro with the human liver tumor cell line, HepG2. *Cell Biol. Toxicol.* 4(3):295-309 (37 refs.).

A human liver tumor cell assay for detecting acute cytotoxicity of polycyclic aromatic hydrocarbons is described. The human hepatocellular tumor cell line HepG2 was used. The authors conclude that the ability of HepG2 cells to detect many chemicals whose toxicity depends upon metabolic transformation makes this assay system preferable to the usual fibroblast assay system.

Borenfreund, E., and H. Babich. 1987. In vitro cytotoxicity of heavy metals, acrylamide, and organotin salts to neural cells and fibroblasts. *Cell Biol. Toxicol.* 3(1):63-73.

The cytotoxicity of neurotoxic agents was determined for a series of brain-derived cell types and compared with their toxic effects on BALB/c 3T3 fibroblasts, using the neutral red assay. The test was sensitive enough to detect structure activity relationships between the degree of toxicity and the hydrophobic characteristics of the agents tested.

- Ha, D. K. K., and W. H. Lau. 1989. Effect of anticancer drugs and hyperthermia on nasopharyngeal carcinoma cell line in vitro. *Cancer Lett.* (Shannon, Ireland) 45(2): 103--107.
The therapeutic effects of 10 anticancer drugs on a cultured nasopharyngeal carcinoma (NPC) cell line were examined by a tritiated thymidine incorporation-inhibition assay and a growth inhibition assay by a microphotometric method. Combined hyperthermia and actinomycin D or vincristine sulfate are markedly cytotoxic for the NPC cells in vitro.
- Kemp, R. B., D. M. Cross, and R. W. Meredith. 1988. Comparison of cell death and adenosine triphosphate content as indicators of acute toxicity in vitro. *Xenobiotica* 18(6):633-639.
Anchorage-independent LS cells, derived from L929 mouse fibroblasts, were used as an in vitro alternative to animals for the assessment of acute toxicity. The two end points were cell death, indicated by fluorescein diacetate and ethidium bromide, and intracellular adenosine triphosphate (ATP) content. Twenty compounds were tested for concentrations that produced a 50 percent increase in ATP content (ATP50) and concentrations that caused 50 percent cell death (CD50). There was a good numerical correlation ($r = 0.99$) between the ranks of ATP50 and CD50 end points. However, the slopes of the dose-response plots for individual chemicals were markedly different.
- Malomi, W., G. Formisano, and F. Iosi. 1988. In vitro effects of styrene on cytoskeletal apparatus: An immunocytochemical study. *Drug Chem. Toxicol.* 11(4):419-431.
The effects of styrene on the cytoskeletal apparatus involved in several toxic neuropathies were studied. The epithelial cell lines, CG5 and HEp-2, were considered as living systems useful for investigating the mechanisms of cytotoxicity. Preliminary results reported here were obtained on these two different epithelial cell lines by immunocytochemical methods. Data obtained seem to confirm in vitro studies as a useful tool in toxicity assessment of xenobiotic compounds at subcellular levels.
- Stratford, I. J., and M. A. Stephens. 1989. The differential hypoxic cytotoxicity of bioreductive agents determined in vitro by the MTT assay. *Int. J. Radiat. Oncol. Biol. Phys.* 16(4):973-976.
Good agreement was obtained between the MIT assay and conventional clonogenic assays regarding the concentration and contact time required to produce a given level of killing of Chinese hamster V79 cells treated in either air or nitrogen with a range of bioreductive cytotoxic drugs. The MTT assay was carded out with V79 cells attached to the bottom of 1-cm glass wells within a 24-well plate. All procedures, that is, drug exposure, cell growth, and metabolism of MTT, were then carded out in situ. It is proposed that this method provides a simple, rapid procedure for evaluating the cytotoxicity of bioreductive drugs.
- Williams, P. P. 1989. Effects of T-2 mycotoxin on gastrointestinal tissues: A review of in vivo and in vitro models. *Arch. Environ. Contam. Toxicol.* 18(3):374--387.
Agricultural products and food are frequently contaminated with T-2 mycotoxin. Various animal models have been used to determine its metabolic fate, rate of excretion, and distribution. A host of bioassay systems are now being used as alternative methods to the use of animals for testing of the mycotoxin. These tests may accurately assess and define the role of the subject-toxin interactions following consumption of T-2 mycotoxin-contaminated food sources. T-2 mycotoxin, as observed in in vivo and in vitro models, promotes a chemically induced change in structure and function of affected gastrointestinal cells from a transient and reversible aberration in a single enzymatic reaction to cell death.
- Yang, B., J. Wan, J. Mi, S. Zhang, and J. Yin. 1988. Comparison of four cytotoxic assays in vitro and in vivo by using the murine leukemia L7811-85 cell line. *Zhongguo Yaolixue Yu Dulixue Zazhi* 2(4):300-302.
This paper, written in Chinese, reports the use of the murine leukemia L7811-85 cell line to assess three in vitro tests for assaying the effectiveness of four antitumor agents. The three in vitro tests are the dye exclusion test, the clonogenic assay, and the 3H-thymidine incorporation assay. An in vivo test was also conducted by determining the increase of survival time of mice inoculated with L7811-85 cells. Results from the in vitro tests were consistent with those from the in vivo test; thus, all three in vitro tests are useful for studying the effectiveness of antitumor agents.

Dental

- al-Nazhan, S., G. Sapounas, and L. Spangberg. 1988. In vitro study of the toxicity of a composite resin, silver amalgam, and cavit. *J. Endod.* 14(5):236-238.

Cheung, H. S., and M. H. Haak. 1989. Growth of osteoblasts on porous calcium phosphate ceramic: An in vitro model for biocompatibility study. *Biomaterials* 10(1):63-67.

Biomaterial implantation in animals is commonly used for biocompatibility studies as well as examination of long-term interaction between tissue and the test material. An in vitro cell culture model is proposed as an alternative that will save animal lives and reduce the pain and discomfort of animals used for such studies. In this study the biomaterial was matched to the cell types typical of the implant site of the particular material: porous calcium phosphate ceramic, used as dental and orthopedic implants, with periosteal fibroblasts, osteoblasts, and chondrocytes. All three cell types attached to the ceramic and formed multicellular layers.

Craig, R. G., and C. T. Hanks. 1988. Reaction of fibroblasts to various dental casting alloys. *J. Oral Pathol.* 17(7):341-347.

The cytotoxicity of a series of dental casting alloys in the as-cast and polished condition was determined with cell culture techniques involving phase contrast microscopy to examine cell morphology and the succinic dehydrogenase histochemical reaction to measure any ring of inhibition of BALB/c 3T3 cellular respiration around alloys. This direct contact method appeared satisfactory for evaluating biocompatibility of dental casting alloys, especially because these materials are in contact with gingival tissues.

Hanks, C. T., R. G. Craig, M. L. Diehi, and D. H. Pashley. 1988. Cytotoxicity of dental composites and other materials in a new in vitro device. *J. Oral. Pathol.* 17(8):396-403.

Several in vitro methods have been developed over the years to improve correlation between screening tests and usage tests of dental restorative materials. In this study an in vitro pulp chamber device is described in which dentin disks were interspersed between composite resins (as well as other dental materials) and the medium that served as the nutrient source for the BALB/c 3T3 test cell system. This system is used to determine the relative cytotoxicity of dental composites and other materials.

Kasten, F. H., L. F. Pineda, P. E. Schneider, H. R. Rawks, and T. A. Foster. 1989. Biocompatibility testing of an experimental fluoride releasing resin using human gingival epithelial cells in vitro. *In Vitro Cell Dev. Biol.* 25(1):57-62.

This study compared the in vitro biocompatibility of an experimental fluoride composite resin with fluoride and nonfluoride-releasing materials currently available. Several dental materials were tested. The Smulow-Glickman (S-G) human gingival epithelial cell line, which exhibits semidifferentiated characteristics, was used in the study as a test system. Biocompatibility was quantified by counting the viable cells per unit area remaining after 24 and 48 hours at two radial distances from cured specimens immersed in the cell culture medium.

Spangberg, L., and E. A. Pascon. 1988. The importance of material preparation for the expression of cytotoxicity during in vitro evaluation of biomaterials. *J. Endod.* 14(5):247-250.

Developmental Toxicology

Steele, V. E., R. E. Morrissey, E. L. Elmore, D. Gurganus-Rocha, B. P. Wilkinson, R. D. Curren, B. S. Schmetter, A. T. I-Louie, J. C. Lamb, and L. Yang. 1988. Evaluation of two in vitro assays to screen for potential developmental toxicants. *Fundam. Appl. Toxicol.* 11(4):673-684.

To evaluate two in vitro assays for their ability to detect known developmental toxicants and nontoxicants, a series of 44 coded compounds were assayed by two independent laboratories using standardized protocols. The two test systems were (1) the human embryonic palatal mesenchymal cell growth inhibition assay and (2) the mouse ovarian tumor cell attachment inhibition assay. The data indicate that the two assays are complimentary, and as such, the combination of these assays could be useful as a preliminary screen to establish priorities for in vivo development toxicity testing.

DNA Effects

Butterworth, B. E., T. Smith-Oliver, L. Earle, D. J. Loury, R. D. White, D. J. Doolittle, P. K. Working, R. C. Cattley, R. Irtle, G. Michalopoulos, et al. 1989. Use of primary cultures of human hepatocytes in toxicology studies. *Cancer Res.* 49(5): 1075-1084.

To examine the genotoxic potential of chemicals in human liver cells, a human hepatocyte DNA repair assay was defined. Procedures were optimized to prepare primary cultures of human hepatocytes from discarded surgical

material. Chemically induced DNA repair, measured as unscheduled DNA synthesis, was quantitated autoradiographically. For purposes of comparison, all test chemicals were evaluated in the in vitro rat hepatocyte DNA repair assay. Individual-to-individual variation in the DNA repair response was far greater for the human cultures than for cultures derived from rats. These results indicate that, in general, the in vitro rat hepatocyte DNA repair assay is a valid model for predicting potential genotoxic effects in human beings.

Nakano, H., N. Saijo, K. Nakagawa, Y. Sasaki, Y. Fuji-wara, K. Minato, M. Bungo, K. Nasahara, and W. S. Hong. 1989. Application of miniaturized improved nucleic acid precursor incorporation assay to in vitro evaluation of new drugs. *Cancer J.* 2(8):267-270.

This study was conducted to investigate the optimal conditions for the miniaturized improved nucleic acid precursor incorporation hybrid assay using two established human lung cancer cell lines and the success rate using 40 freshly obtained tumor specimens. Using this assay system, the authors have studied the antitumor activity of two new anticancer agents, FK973 and 254S, against freshly obtained specimens and cultured cell lines of nonsmall cell lung cancer.

Sondak, V. K., E. L. Korn, and D. H. Kern. 1988. In vitro testing of chemotherapeutic combinations in a rapid thymidine incorporation assay. *Int. J. Cell Cloning* 6(6):378-391.

A total of 199 solid human tumors, of several types, were tested with a rapid thymidine incorporation assay for sensitivity to one of several clinically used multidrug combinations and to each agent in the combination separately. From the results of these tests, it is concluded that the rapid thymidine incorporation assay can be used to test for in vitro sensitivity to drug combinations and that sensitivity to a drug combination can be inferred if a tumor is sensitive to any component drug of the combination.

Turina, M. P., A. Colacci, S. Grilli, M. Mazzullo, G. Prodi, G. Lattanzi, B. Bonora, S. Bartoli, and L. Guidotti. 1989. Metabolic activation and covalent binding to nucleic acids of pentachloroethane as short-term test of genotoxicity. *Res. Commun. Chem. Pathol. Pharmacol.* 63(1):81-91.

The in vivo covalent binding of ¹⁴C-pentachloroethane to DNA, RNA, and proteins of rats and mouse organs was detected 22 hours after intraperitoneal injection. The binding extents obtained from in vitro incubation and the binding values detected after in vivo administration of labeled pentachloroethane were comparable and showed a high correlation with the oncogenic potency index of this compound. This result confirms the efficiency of in vitro binding as a short-term test of genotoxicity prediction.

Yoshimi, N., S. Sugie, H. Iwata, K. Niwa, H. Mori, C. Hashida, and H. Shimizu. 1988. The genotoxicity of a variety of aniline derivatives in a DNA repair test with primary cultured rat hepatocytes. *Mutat. Res.* 206(2): 183-191 (40 refs.).

The genotoxicity of aniline derivatives was studied in vitro. Genotoxicity was assessed by determining the extent of DNA repair. The extent of DNA repair was determined as the amount of unscheduled DNA synthesis by measuring uptake of radiolabeled thymidine. The data were compared with previously obtained mutagenesis and carcinogenesis data.

Endocrinology

Dahl, K. D., H. Papkoff, and A. J. W. Hsueh. 1989. Effects of diverse mammalian and nonmammalian gonadotropins in a rat granulosa cell bioassay for follicle-stimulating hormone. *Gen. Comp. Endocrinol.* 73(3):368-373.

The biopotencies of pituitary gonadotropins from several mammalian and nonmammalian species were examined using an in vitro rat granulosa cell bioassay for follicle-stimulating hormone (FSH). Treatment of cultured granulosa cells with increasing concentrations of gonadotropin preparations from these species resulted in dose-dependent increases in estrogen production. This in vitro bioassay using rat granulosa cells provides a sensitive and specific assay for measuring FSH activities of gonadotropins from diverse mammalian and non-mammalian species.

Enzymology

Reitz, R. H., A. L. Mendtala, C. N. Park, M. E. Andersen, and F. P. Guengerich. 1988. Incorporation of in vitro enzyme data into the physiologically-based pharmacokinetic (PB-PK) model for methylene chloride: Implications for risk assessment. *Toxicol. Lett.* 43(1-3):97-116.

Physiologically based pharmacokinetic (PB-PK) models provide a mechanism for reducing the uncertainty inherent in extrapolating the results of animal toxicity tests to humans. This paper discusses a technique for incorporating data from in vitro studies of xenobiotic metabolism into in vivo PB-PK models. Methylene chloride is used as an example, and carcinogenic risk estimates incorporating PB-PK principles are presented.

Eye

Jackson, E. M., R. D. Hume, and R. F. Wallin. 1988. The agarose diffusion method for ocular irritancy screening: Cosmetic products, part H. *J. Toxicol., Cutaneous Ocul. Toxicol.* 7(3): 187-194.

Twenty-two cosmetic products were tested by the agarose diffusion method to determine the correlation between the zone of lysis produced by agarose diffusion and the 24-hour rating of chemical irritation in previously conducted Draize eye irritancy tests using these same formulations. A 100 percent correlation was established for this group of samples between the in vitro agarose diffusion test and the in vivo Draize eye irritancy test, with no false-positive or false-negative results.

Kato, S., H. Itagaki, I. Chiyoda, S. Hagino, T. Kobayashi, Y. Fujiyama, H. Kakoki, and M. Yamaguchi. 1988. Liposomes as an in vitro model for predicting the eye irritancy of chemicals. *Toxicol. In Vitro* 2(2):125-130. Liposomes containing 4-methylumbelliferyl phosphate (Um-P) were prepared using the lipid extracts from bovine eyes and were incubated with seven surface-active agents. The Um-P released from the liposomes by each test agent was hydrolysed with alkaline phosphatase, and the resultant 4-methylumbelliferone was assayed spectrofluorometrically. The values for Um-P50 (the concentration of test material at which 50 percent of Um-P is released) showed a good inverse correlation with the irritation scores obtained by the Draize eye test.

Kelly, C. P. 1988. In vitro method of predicting ocular safety. Part 1. *Drug Cosmet. Ind.* 143(Aug.):34, 36, 38, 70 (4 refs.).

Research and standardization in developing an in vitro method of predicting ocular safety of chemicals are discussed, including a scale of weighted scores for grading the severity of ocular lesions.

Kelly, C. P. 1988. In vitro method of predicting ocular safety. Part 2. *Drug Cosmet. Ind.* 142(Sept):54, 59-60, 62, 64, 112 (7 refs.).

The development of Eytex, a newly proposed system for standardizing ocular irritation testing, is described. Published results demonstrate the efficacy and usefulness of the new method and detail the high degree of correlation with the results of the Draize in vivo method.

Kennah II, H. E., D. Albulescu, S. Hignet, and C. S. Barrow. 1989. A critical evaluation of predicting ocular irritancy potential from an in vitro cytotoxicity assay. *Fundam. Appl. Toxicol.* 12(2):281-290.

This study evaluated the use of in vitro cytotoxicity data for predicting the ocular irritancy potential of 24 chemicals in BALB/c 3T3 cells. Although a significant linear correlation between cytotoxicity and ocular irritancy was established for surfactants and alcohols, overall a poor correlation existed between cytotoxicity and ocular irritancy. The lack of correlation illustrates that in vitro cytotoxicity data cannot be used to predict the ocular irritancy potential of a broad spectrum of chemicals.

Koeter, H. B. W., and M. K. Prinsen. 1987. Validation of an in vitro eye irritancy test: A first step. *Altern. Meth. Toxicol.* 5(In Vitro Toxicol.):79-86.

After the introduction of standard criteria for the assessment of effects and consequent classification in the enucleated superfused eye test, a comparative study of this in vitro test system and the in vivo eye irritation test was conducted with 39 substances. From the results it appeared that overestimation of the in vitro test only occurred in 13 percent and underestimation in 7 percent of the tests. Thus, the enucleated superfused eye test is certainly worthy of further study and merits introduction in a number of laboratories for further evaluation.

Shopsis, C., E. Borenfreund, and D. M. Stark. 1987. Validation studies on a battery of potential in vitro alternatives to the Draize test. *Altern. Meth. Toxicol.* 5(In Vitro Toxicol.):31-44.

This publication is a review with 21 references on the development of in vitro alternatives to the Draize test for ocular irritancy.

Hepatotoxicity

Gomez-Lechon, M. J., A. Larrauri, T. Donato, P. Lopez, A. Montoya, and J. V. Castell. 1988. Predictive value of the in vitro test for hepatotoxicity of xenobiotics. *Inst. Natl. Sante Rech. Med. [Colloq.]* 164(Liver Cells Drugs):371-377.

This study describes an integrated series of experiments to evaluate the action of a xenobiotic on the biology and biochemistry of hepatocytes. This screening uses cultured cells, instead of isolated cells, which allows the design of experiments with longer exposures to the xenobiotics. Several facts were taken into consideration: (1) the use of a biological system that should reproduce to a great extent the biochemistry of the liver, (2) the choice of the most appropriate parameters for detecting and quantifying the hepatotoxic (cytotoxic or metabolic) effects of the xenobiotic in culture, and (3) the predictive value of the data obtained in vitro in relation to the expected and/or observed toxicity in vivo.

Gomez-Lechon, M. J., A. Montoya, P. Lopez, T. Donato, A. Larrauri, and J. V. Castell. 1988. The potential use of cultured hepatocytes in predicting the hepatotoxicity of xenobiotics. *Xenobiotica* 18(6):725-735.

A protocol is proposed for screening for hepatotoxicity of xenobiotics in vitro in which hepatocytes exposed to the compounds are evaluated for both cytotoxic and metabolic effects.

Guguen-Guillouzo, C., P. Gripon, Y. Vandenberghe, F. Lamballe, D. Ratanasavanh, and A. Guillouzo. 1988. Hepatotoxicity and molecular aspects of hepatocyte function in primary culture. *Xenobiotica* 18(6): 773-783 (71 refs.).

The application of primary cultures of hepatocytes in testing for hepatotoxicity of drugs is reviewed. Coculturing hepatocytes with primitive biliary cells prolongs cell survival and their functional capacities for several weeks, including some of the transcriptional activity.

Ratanasavanh, D., G. Baffet, M. F. Latinier, M. Rissel, and A. Guillouzo. 1988. Use of hepatocyte cocultures in the assessment of drug toxicity from chronic exposure. *Xenobiotica* 18(6):765-771.

Results presented here suggest that cocultured hepatocytes that retain their differentiated state for several days or weeks represent a promising tool for studying hepatotoxicity from chronic treatment in vitro.

Tyson, C. A. 1987. Correspondence of results from hepatocyte studies with in vivo response. *Toxicol. Ind. Health* 3(4):459-478 (87 refs.).

Isolated hepatocyte cultures as a means of evaluating systemic chemical toxicity are discussed, and conditions for isolating and maintaining hepatocyte cultures are summarized. In vitro studies with several compounds and some classes of compounds are considered, and comparisons are made with results obtained in vivo.

Comparisons are made with results from studies using hepatocytes from several animal species. A scheme for determining hepatotoxic potential in isolated hepatocyte cultures is presented.

Immunology

DeLeo, V., J. Hong, S. Scheide, B. Kong, S. DeSalva, and D. Bagley. 1988. Surfactant-induced cutaneous primary irritancy: An in vitro model-assay system development. *Altern. Meth. Toxicol.* 6 (Prog. In Vitro Toxicol.):39-43.

Using human epidermal keratinocytes in culture as an in vitro model system, it was shown that the irritant process involves surfactant-induced stimulation of phospholipases. This paper describes an effort to use this mechanism of phospholipase activation as an assay for irritancy. Cells in culture were prelabeled with either 3H-arachidonic acid or 3H-choline. The cells were treated with five different finished products of known irritancy in animal skin (Primary Dermal Irritancy Index-PDII). Release of radiolabel was assayed, and the concentration of product necessary to induce release at 150 percent of control was calculated (ID 150 percent). The rank order of the PDII and ID 150 percent correlated fairly well for the five products. In addition the human keratinocyte line (NHEK-Clonetics) was used in release assays, and the response of these cells to that of primary keratinocyte cultures showed an excellent correlation.

Hendrikseu, C. F., J. W. van der Gun, J. Nagel, and J. G. Kreeftenberg. 1988. The toxin binding inhibition test as a reliable in vitro alternative to the toxin neutralization test in mice for the estimation of tetanus antitoxin in human sera. *J. Biol. Stand.* 16(4):287-297.

A method for the screening of human sera for tetanus antibodies has been developed and evaluated. The toxin binding inhibition test (ToBI test) is based on inhibition of the binding of tetanus toxin to an antitoxin-coated immunoassay microtitre plate by tetanus antibodies. After testing serum samples from 191 healthy adults with

different vaccination histories, it is concluded that the ToBI test is a reliable and precise alternative to the toxin neutralization test in mice and can be performed under simple laboratory conditions in a short time.

Hendriksen, C. F., J. W. van der Gun, and J. G. Kreeftenberg. 1989. Combined estimation of tetanus and diphtheria antitoxin in human sera by the in vitro toxin-binding inhibition (ToBI) test. *J. Biol. Stand.* 17(2): 191-200. A toxin-binding inhibition (ToBI) test was developed that could be used for a combined estimation of both tetanus and diphtheria antitoxin levels. The application of streptavidin-biotinylated peroxidase complex when using small serum samples is discussed. Sera from 140 adults with different vaccination histories were titrated for both tetanus and diphtheria antitoxin. Good correlations were found between the estimates obtained by the ToBI test and those obtained by the toxin-neutralization (TN) test in mice (tetanus antitoxin) and those obtained by the in vitro neutralization test in VERO cells (diphtheria antitoxin). It is concluded that the ToBI test is a simple and reliable alternative to the functional models currently in use for the estimation of diphtheria and tetanus antitoxin levels. In addition, the ToBI test eliminates the need for laboratory animal or cell culture facilities and can be performed with small quantities of serum.

Robinson, M. K. 1989. Optimization of an in vitro lymphocyte blastogenesis assay for predictive assessment of immunologic responsiveness to contact sensitizers. *J. Invest. Dermatol.* 92(6):860--867. Predictive animal tests, generally using guinea pigs, require a relatively large number of animals to produce a sufficient database for interpreting skin reaction scores. In vitro assays have the potential of being more quantitative than skin testing and, d so, would require fewer animals. An in vitro lymphocyte blastogenesis assay has been optimized for detecting the response of mouse lymphocytes to strong contact sensitizers, with the eventual objective of applying this assay to moderate and weak sensitizers as well. Results presented in this publication represent an attempt to directly modify cultured epidermal cells with oxazolone and use these cells to stimulate oxazolone-directed blastogenesis in microtitre plate cultures. This optimized assay is now under evaluation for predictive assessment of contact sensitizers relevant to occupational and consumer exposures.

Immunotoxicity

Comacoif, J. B., A. N. Tucker, and J. H. Dean. 1988. Development of a human peripheral blood mononuclear leukocyte model for immunotoxicity evaluation. *In Vitro Toxicol.* 2(2):81-90. T-cell function was assessed in a one-way mixed lymphocyte culture assay developed using human peripheral blood mononuclear leukocytes (PBMLs) and a cryopreserved pool of stimulator lymphocytes. An enzyme-linked immunosorbant assay (F-LISA) for immunoglobulin G produced in response to pokeweed mitogen was evaluated for the assessment of B-cell function. These assays were examined as potential biomarkers for immune dysfunction induced in vitro by 7,12-dimethylbenz(a)anthracene. The data demonstrate that the assay parameters selected may provide appropriate biomarkers for detecting T and B cell dysfunction following in vitro exposure to highly lipophilic polycyclic aromatic hydrocarbons. The study of human PBMLs may become an important adjunct to clinical immunotoxicity assessment and may provide valuable information regarding risk extrapolation in humans.

Irritancy

Brown, S., L. Templeton, D. A. Prater, and C. J. Potter. 1989. Use of an in vitro haemolysis test to predict tissue irritancy in an intramuscular formulation. *J. Par-enter. Sci. Technol.* 43(3): 117-120. As part of a drug development program, an in vitro hemolysis test was used to predict the in vivo muscle tissue irritancy of intramuscular formulations of a new chug entity. The results indicated that the test was able to differentiate formulations in terms of hemolytic potential, and the correlation between the hemolysis test results and in vivo intramuscular irritancy studies indicated that the test may be used to screen formulations for potential tissue irritancy prior to in vivo evaluation.

Javed, S., V. Misra, and P. N. Viswanathan. 1989. In vitro studies on the effect of linear alkylbenzenesulfonate on serum albumin, *Int. J. Cosmet. Sci.* 10(5):241-246.

Linear alkylbenzenesulfonate was found to influence the thermal and fluorescence profile of serum albumin in vitro, which indicates the possibility of protein-detergent interaction in the skin irritancy of detergents.

Lung

Orefro, V. I. C., D. Thomas, and R. J. Richards. 1988. Comparative toxicity studies on cultured alveolar epithelial type H cells and lung fibroblasts exposed to agents that induce pulmonary edema. *Chim. Oggi* (ISS 6):57-61. A number of agents with the potential to produce pulmonary edema were examined for toxic effects on cultures of isolated alveolar epithelial type II cells and lung fibroblasts. Determination of toxicity was made using a rapid, quantitative dye (neutral red) accumulation assay and by comparing the concentration of agents required to produce a TD50 (reduction of dye accumulation to 50 percent that observed in untreated cultures). For a number of lung-damaging compounds, the type II cell culture provides a rapid screening process, the means to examine the mechanism of cell damage, and perhaps the potential to investigate the effects of toxins on type I cells in culture.

Riebe, M., M. Emura, M. Aufderheide, and U. Mohr. 1988. Target organ cells as in vitro test systems for toxicity, mutagenicity, DNA-repair, and transformation by environmental pollutants. *Environ. Hyg. [Eur. Meet. Environ. Hyg.]* 1:49-53.

A cell line, established from the fetal Syrian golden hamster lung, and following application of different culture conditions, differentiated into certain types of epithelial cells present in the respiratory tract in vivo. The cell line M3E3/C3 is proposed as a target organ cell system for the testing of cyto- and genotoxic as well as transforming chemicals relevant in inhalation toxicology.

Metabolism

Reitz, R. H., A. L. Mendtala, and F. P. Guengerich. 1989. In vitro metabolism of methylene chloride in human and animal tissues: Use in physiologically based pharmacokinetic models. *Toxicol. Appl. Pharmacol.* 97(2):230--246. This paper reports studies designed to estimate the in vivo rates of metabolism of methylene chloride (CH₂Cl₂) from in vitro incubations of lung and liver tissues from B6C3F1 mice, F344 rats, Syrian golden hamsters, and humans. A procedure for calculating in vivo metabolic rate constants from the in vitro studies is presented. This procedure was validated by making extrapolations with mixed function oxidase enzymes acting on CH₂Cl₂, where both in vitro and in vivo rates of metabolism are known. When in vivo studies in humans are unavailable, in vitro enzyme assays provide a reasonable method for estimating metabolic rate constants.

Watson, W. P., R. J. Smith, K. R. Huckle, and A. S. Wright. 1989. Use of organ cultures in human risk assessment: Comparisons of benzo(a)pyrene DNA adducts in mouse and human skin. *Toxicol. In Vitro* 3(1):69-76. Human and mouse skin explant systems have been developed as models that qualitatively mimic the genotoxic metabolism in vivo of benzo(a)pyrene, a representative carcinogenic polycyclic aromatic hydrocarbon. This publication presents approaches that have been applied in order to develop valid risk models for chemical carcinogens and to provide valuable reference standards for the detection of human genotoxic metabolism. The coupling of human skin explant systems with ³²P- postlabeling techniques has provided a direct in vitro model for the prospective detection of human carcinogens.

Methodology

Ennever, E K., and H. S. Rosenkranz. 1988. Methodologies for interpretation of short-term test results which may allow reduction in the use of animals in carcinogenicity testing. *Toxicol. Ind. Health* 4(2): 137-149 (35 refs.). Assessment of the risk to humans posed by chemical substances currently relies primarily on experimental exposure of animals in lifetime feeding studies. Short-term tests for genotoxicity are much less costly and use fewer or no animals, but have not replaced the long-term animal bioassay because their results do not coincide completely. Methodologies are described for interpretation of short-term tests that improve the usefulness of their results, and may allow them to replace the long-term animal bioassay in some circumstances.

Hu, S. L., Y. Y. Haimes, and R. S. Galen. 1988. Optimal selection of a battery of tests: A multiobjective optimization methodology. *Med. Decis. Making* 8(1):19-32.

The general problem of selecting a battery of tests for diagnostic purposes is discussed, and multiobjective optimization methodology is applied to solve it, with battery selection being based on performance indices such as sensitivity, specificity, and the cost of testing. The major advantage of the model developed in this paper is that it can generate a set of noninferior batteries without requiring the calculation of all possible combinations of tests. An example in which the method is applied to a real problem--the selection of short-term tests to detect the carcinogenicity of chemicals---is discussed.

Moslen, M. T., and M. S. Legator. 1988. Advantages of a combined testing protocol (CTP) with multiple end points: The reverse tier approach. *Mutat. Res.* 205(1--4):91-97.

A multiple end point CTP approach provides an efficient procedure to investigate the hazards of chemicals in our environment. Genotoxicity to multiple tissues is assessed after an exposure of limited duration by using a variety of sensitive short-term tests. Numbers of animals required to test chemicals are substantially decreased by increasing the amount of information obtained from each animal.

Mutagenesis

Casparly, W. J., D. S. Daston, B. C. Myhr, A. D. Mitchell, C. J. Rudd, and P. S. Lee. 1988. Evaluation of the LS178Y mouse lymphoma cell mutagenesis assay: Interlaboratory reproducibility and assessment. *Environ. Mol. Mutagen.* 12(S 13):195-229 (69 refs.).

The LS178Y mouse lymphoma cell mutagenesis assay was used to evaluate interlaboratory reproducibility and assessment in the detection of the mutagenic activity of 63 chemicals in a mammalian cell system. The authors conclude that the mouse lymphoma cell forward mutation assay detects chemical mutagenicity in a manner that is consistent with other genetic end points as well as rodent carcinogenicity studies.

Chang, G., D. Jacobson-Kram, and J. R. Williams. 1988. Use of an established human hepatoma cell line with endogenous bioactivation for gene mutation studies. *Cell Biol. Toxicol.* 4(3):267-279 (32 refs.).

A gene mutation assay using an established human hepatoma cell line was described. The assay was based on inducing 6-thioguanine (TG)-resistant mutants in the human hepatoma cell line HepG2. The authors conclude that the HepG2 cell line is useful for screening potential human mutagens.

Nephrotoxicity

Bach, P. H. 1989. Detection of chemically induced renal injury: The cascade of degenerative morphological and functional changes that follow the primary nephrotoxic insult and evaluation of these changes by in vitro methods. *Toxicol. Lett.* 46(1-3):237-249 (37 refs.).

A diversity of chemicals causes discrete lesions in the kidney by a number of different mechanisms, and similar types of chemicals may give rise to more than one target cell injury. In vitro techniques can provide answers to specific questions about the mechanisms by which chemicals damage these discrete cell types. It is essential that a number of different in vitro systems be developed in parallel to properly address the mechanistic aspects of and screening for nephrotoxicity. Data generated in vitro must be related to the situation in vivo and used to devise reliable noninvasive tests for assessing nephrotoxicity in humans.

Bach, P. H., and E. N. Kwizera. 1988. Nephrotoxicity: A rational approach to target cell injury in vitro in the kidney. *Xenobiotica* 18(6):685-698 (112 refs.).

Discrete renal injury can be exploited in vitro to study the interactions between the toxic compound and the target cell. Several in vitro models have been used to study the potential interaction between the target cells and chemicals. The target cell toxicity in vivo of adriamycin, 2-bromoethanamine, and hexachlorobutadiene N-acetyl cysteine conjugate is selectively maintained towards glomerular epithelial, medullary interstitial, and proximal tubular cells, respectively, in vitro, which shows that the "in vivo-in vitro gap" can be bridged.

Bradley, M. O., C. Noble, and J. E. Sina. 1989. An in vitro nephrotoxicity assay useful in drug development. *In Vitro Toxicol.* 2(3): 171 - 182.

An in vitro assay is described for measuring toxicity to proximal tubule cells isolated from rabbit kidneys. The assay was used for predicting the relative nephrotoxicity of compounds having similar structure and thus can be useful in selecting a promising compound from others of unknown toxicity but known pharmacologic activity. The in vitro nephrotoxicity assay can also be used for studying cellular mechanisms of toxicity. Other applications for the assay include helping to determine the basis for species-specific toxicity of given compounds and also the basis for organ-specific toxicity.

Neurology

Coleman, R. A., and A. T. Nials. 1989. Novel and versatile superfusion system. Its use in the evaluation of some spasmogenic and spasmolytic agents using guinea-pig isolated tracheal smooth muscle. *J. Pharmacol. Methods*

21(1):71-86.

An eight-chamber superfusion system that is suitable for a variety of applications involving the study of both contraction and relaxation of smooth muscle preparations, and the effect of agents that interfere with these actions, was developed. The system allows electrical stimulation of preparations, and thus neuronally mediated responses and agents that interfere with neurotransmission may also be studied. To demonstrate some of the applications of the system, an evaluation was made of both spasmogenic and spasmolytic agents on an isolated guinea pig tracheal strip preparation. This superfusion system is described as having several advantages over previously described superfusion or immersion techniques.

Neurotoxicity

Khera, K. S., and C. Whalen. 1988. Detection of neuroteratogens with an in vitro cytotoxicity assay using primary monolayers cultured from dissociated fetal rat brains. *Toxicol. In Vitro* 2(4):257-274.

Cells from fetal rat brain tissue were seeded and grown in a monolayer obtained after incubation for three days. The layer consisted of two readily distinguishable cell types: neuronal cells with interconnecting neurites and fascicles, and nonneuronal or glial-type cells. The cell layers were exposed to graded concentrations of a test chemical dissolved in nutrient medium. The cultures were examined daily for cell death and inhibited outgrowth of neurites and fascicles for three days, and the test was then terminated. The agreement between the neuroteratogenic potential in vivo, as reported in the literature, and the cytotoxicity data obtained in this study was very good for 82 chemicals, reasonable for 10, poor for 4, and undetermined for 4. From the results of this investigation and a review of the published teratology data, the neuron and its precursor neuroepithelial cell appear to be the most likely initial targets for neuroteratogenic chemicals. Consequently, this in vitro system offers a promising approach for studies on neuroteratogens.

Ogura, A., M. Miyamoto, and Y. Kudo. 1988. Neuronal death in vitro: Parallelism between survivability of hippocampal neurons and sustained elevation of cytosolic calcium after exposure to glutamate receptor agonist. *Exp. Brain Res.* 73(3):447-458.

Hippocampal neurons isolated from rat embryos were maintained on glial monolayers in a medium containing no L-glutamate (Glu). The administration of Glu for a limited period induced a massive death (loss) of neurons. An increase in concentration of external Ca^{2+} during the exposure to Glu enhanced the extent of loss. By contrast, an increment in concentration of environmental Mg^{2+} reduced the loss. The findings presented here show that neuronal death resulting from an extraneous excitation (excitotoxicity) can be analyzed in vitro.

Skin

Bagley, D. M., J. Blankson, B. M. Kong, and S. J. De Salva. 1988. Optimization and evaluation of an in vitro yeast assay to predict phototoxicity. *J. Toxicol. Cutaneous Ocul. Toxicol.* 7(4):295-308.

Phototoxicity is a skin-cell damage response that follows exposure, in many instances, to a juxtapositional combination of certain chemicals and ultraviolet A (UVA) light. Many known phototoxins are fragrances or components of fragrances. Because of the commercial value of fragrance materials to household and personal care products, it is important to assess their phototoxic potential. To meet this objective and to minimize the use of animals, a modified in vitro yeast assay was used. Results using this modified assay correlated well with in vivo data.

Carver, M. P., P. L. Williams, and J. E. Riviere. 1989. The isolated perfused porcine skin flap. III. Percutaneous absorption pharmacokinetics of organophosphates, steroids, benzoic acid, and caffeine. *Toxicol. Appl. Pharmacol.* 97(2):324-337.

The isolated perfused porcine skin flap (IPPSF) has been developed as an alternative in vitro tool for examining the pharmacokinetics and mechanisms of percutaneous absorption. Results described here suggest that xenobiotic penetration in eight-hour IPPSF experiments is highly predictive of in vivo absorption totals (six-day studies). Because pig and human skin are similar physiologically and pharmacologically, the IPPSF may eventually have applications in formulating human dermal risk assessment models.

Ferguson, J., M. W. Brown, A. W. Hubbard, and M. I. Shaw. 1988. Determination of sun protection factors. Correlation between in vivo human studies and an in vitro skin cast method. *Int. J. Cosmet. Sci.* 10(3):117-129. Sun protection factors (SPFs) were determined by an in vitro method that used resin casts taken from replicas of human skin and by an in vivo SPF method. Product development samples (38) were tested for the level of sun

protection using both methods, and the results showed a positive relationship. The cast technique is quick, convenient, inexpensive, and in its present form useful for screening sunscreen products prior to in vivo SPF testing.

Guzman, A. D., S. O. Diez, and D. M. Herraiez. 1988. Determination of parameters of cutaneous permeability in vitro. Part 1. Alkylanilines *Cienc. Ind. Farm.* 7:275-280 (23 refs.).

A method to study percutaneous absorption in vitro is described, and the parameters that define the model of penetration of a homologous series of 4-substituted anilines through full thickness Wistar rat skin are determined.

Houk, J., and R. H. Guy. Research in Progress. Membrane Models for Skin Penetration Studies. Departments of Pharmaceutical Chemistry and Pharmacy, Schools of Pharmacy and Medicine, University of California, San Francisco, California, Grant No. K01-OH-00017, 67 pages, 90 refs.

The results of research efforts into membrane models for skin penetration are reviewed. Eggshell membrane, a membrane composed of synthetic zeolites incorporated into a polystyrene matrix and some simple organic liquids have been proposed as models for biological membranes.

Oliver, G. J., M. A. Pemberton, and C. Rhodes. 1986. An in vitro skin corrosivity test--modifications and validation. *Food Chem. Toxicol.* 24(6-7):507-512.

Initial validation of an in vitro rat epidermal slice technique with 63 chemicals resulted in a high sensitivity for corrosive chemicals but a lower specificity for irritant chemicals. Subsequent modification relating to chemical contact resulted in an improved specificity (i.e., fewer false positives) at the expense of a small loss in sensitivity (i.e., an increase in the number of false negatives). An intralaboratory double blind trial with 34 corrosive chemicals and 36 irritants showed the technique to have total sensitivity (i.e., no false negatives) and a specificity of 88 percent. The results of the initial validation and the double blind trial illustrate the robust nature and high reproducibility of this in vitro technique for identifying skin-corrosive chemicals.

Oliver, G. J. A., M. A. Pemberton, and C. Rhodes. 1988. An in vitro model for identifying skin-corrosive chemicals: I. Initial validation. *Toxicol. In Vitro* 2(1):7-18.

An in vitro epidermal slice technique was developed for identifying chemicals with potential to cause a corrosive lesion in animal skin in vivo. Skin-corrosive potential has been correlated with ability to reduce the skin's penetration barrier by lysis of the stratum corneum. This effect was measured as a lowering of electrical resistance of an epidermal slice following chemical contact in vitro. Initial validation with 68 chemicals showed the technique to have a high sensitivity for corrosive chemicals. The model has potential as a prescreen for conventional animal tests and provides quantitative and objective data-

Shah, V. P., J. Elkins, S. Y. Lam, and J. P. Skelly. 1989. Determination of in vitro drug release from hydrocortisone creams. *Int. J. Pharm.* 53(1):53-59.

A method for the determination of in vitro release of hydrocortisone from creams using Franz diffusion cell system and synthetic membranes was developed. The use of synthetic membrane minimizes the variability observed with skin membranes. The method can be used as a quality control procedure for assuring batch-to-batch uniformity of topical products.

Young, J. R., M. J. How, A. P. Walker, and W. M. H. Worth. 1988. Classification as corrosive or irritant to skin of preparations containing acidic or alkaline substances, without testing on animals. *Toxicol. In Vitro* 2(1): 19-26.

A method is proposed by which substances or preparations may be classified as irritant or corrosive to skin, without being tested on animals, when the irritant or corrosive properties are due to the acidity or alkalinity of the substances or preparations. Results from the application of this approach to a range of commercial cleaning, maintenance, and detergent preparations are in good overall agreement with results based on patch testing in rabbits. Variations in the results obtained from analysis of pH and acid or alkali data were no greater than might be expected from variations in results from animal test methods.

Statistics

Marazzi, A., C. Ruffieux, and A. Randriamiharisoa. 1988. Robust regression in biological assay: Application to the evaluation of alternative experimental techniques. *Experientia* 44(10):857-873.

Robust Huber type regression and testing of linear hypotheses were adapted to statistical analysis of parallel line and slope ratio assays. They were applied in the evaluation of results of several experiments carried out in order to compare and validate alternatives to animal experimentation based on embryo and cell cultures.

Structure Activity

Enslein, K. 1988. An overview of structure-activity relationships as an alternative to testing in animals for carcinogenicity, mutagenicity, dermal and eye irritation, and acute oral toxicity. *Toxicol. Ind. Health* 4(4):479-498. The use of structure-activity relationships (SAR) has proven practical for the development of equations that can be used to estimate end points listed in the title for a large variety of chemicals. The SAR models predict these end points correctly in 85-97 percent of the cases and often surpass in their predictive ability the results obtainable from the equivalent biological assays. Used within proper constraints, SAR models have considerable potential for reducing the number of animals used in toxicity testing.

Rosenkranz, H. S., and G. Klopman. 1988. CASE, the computer-automated structure evaluation system, as an alternative to extensive animal testing. *Toxicol. Ind. Health* 4(4):533-540. CASE, an artificial intelligence system with demonstrated ability to predict biological activity based on structural considerations, correctly predicts animal carcinogenicity. It can, therefore, play a pivotal role in classifying chemicals as carcinogens and priority ranking them for further testing. CASE also shows promise in the design of pharmacologically active agents by reducing the number of drugs that need to be synthesized and tested. For both of these applications, CASE provides a mechanism to conserve animal and other testing resources.

Teratology

Cicurel, L., and B. P. Schmid. 1988. Postimplantation embryo culture: Validation with selected compounds for teratogenicity testing. *Xenobiotica* 18(6):617-624.

Some chemical compounds selected by experts for the validation of in vitro teratogenicity testing were investigated in whole rat embryos cultured during the early stages of organogenesis. All 16 known in vivo teratogens tested also induced specific malformations in embryos grown in culture. The results showed a high predictability of this system for the compounds tested and suggest that the postimplantation embryo culture system may also be useful in the prospective testing of new drugs and environmental chemicals.

Dawson, D. A., D. J. Fort, D. L. Newell, and J. A. Bantie. 1989. Developmental toxicity testing with FETAX: Evaluation of five compounds. *Drug Chem. Toxicol.* 12(1):67-76.

The teratogenic potential of five compounds was evaluated with FETAX (frog embryo teratogenesis assay; *Xenopus*). Early embryos of *Xenopus laevis* were exposed. The results support continued evaluation of FETAX as a screening assay for chemical teratogens.

Johnson, E. M., L. M. Newman, and L. J. Fu. 1989. An in vitro assay for teratogens with cultures of rat embryo midbrain and limb bud cells. *Toxicol. Appl. Pharmacol.* 99(1): 173-180.

Renault, J. Y., C. Melcion, and A. Cordier. 1989. Limb bud cell culture for in vitro teratogen screening: Validation of an improved assessment method using 51 compounds. *Teratogenesis Carcinog. Mutagen.* 9(2):83-96.

Rat embryo limb bud cells multiply and undergo chondrogenesis in micromass culture. Teratogenic agents are identified from their inhibition of chondrogenesis, which is quantified by determination of cartilaginous foci number or proteoglycan production. The improved technique involves simultaneous measurement of cartilage synthesis and cell multiplication. Differentiation was evaluated by measurement, using an Artek Counter, of nodule areas after alcian blue staining, and proliferation by spectrophotometric quantification of crystal-violet bound to micromass cells. The method is fully miniaturized, automated, and computerized, which allows numerous compounds to be rapidly tested at very low cost.

Tsuchiya, T., A. Matuoka, S. Sekita, T. Hisano, A. Takahashi, and M. Ishidate, Jr. 1988. Human embryonic cell growth assay for teratogens with or without metabolic activation system using microplate. *Teratogenesis Carcinog. Mutagen.* 8(5):265-272.

An in vitro microassay for the screening of teratogens was investigated on cancer chemotherapeutic agents sterigmatocystins and benzimidazoles using human embryonic palatal mesenchymal cells. The effects of a metabolic activation system were demonstrated in this microassay.

Toxicity

Frazier, J. M., C. A. Tyson, C. McCarthy, J. J. McCormick, D. Meyer, G. Powis, and L. Ducat. 1989. Potential use

of human tissues for toxicity research and testing. *Toxicol. Appl. Pharmacol.* 97:387-397.

By using in vitro methodologies, the toxicological response of human tissues to xenobiotics can be directly assessed under controlled experimental conditions in advance of human exposure. The object of the symposium presented at the 26th Annual Meeting of the Society of Toxicology was to assess the state of the art and other relevant issues concerning the use of human tissues as in vitro systems for research and testing.

Tyson, C. A., and N. H. Stacey. 1989. In vitro screens from CNS, liver and kidney for systemic toxicity. *Toxicol. Ind. Health* 5(1):107-132 (161 refs.).

The development and evaluation of in vitro systems from target organs for preliminary assessments of the potential for systemic toxic effects has been receiving increased attention. This review presents a synopsis of progress made in developing toxicity screens for three common target organs and identifies further work needed for more complete validation.

Zanini, P. G. 1988. Human trophoblastic tissues cultured in vitro: Evaluation of the basal functionality and function after the administration of potentially toxic substances. *Medicina (Florence)* 8(2):204-206.

This paper, written in Italian, reports the formation and secretion of human chorionic gonadotropin as measured in cultured human trophoblast tissue after treatment with cadmium or methylmercury. The method may be suitable for the study of potential effects of drugs and toxic chemicals on the placenta.

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