ADDITIONAL INFORMATION

Included in this section are additional materials to be considered for the October 17th **International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity**. These materials include:

- ✓ Information pertaining to the 13th Meeting of the Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC), published in *Alternative Testing Methodologies* (*Environmental Health Perspectives*, Volume 106, Supplement 2, April 1998). This special issue of *Environmental Health Perspectives* (edited by W. Stokes, E. Marafante, D. Peakall, and B. Goldstein) summarizes the conclusions of the SGOMSEC Meeting and provides related papers. SGOMSEC, established in 1979, is a nongovernmental organization sponsored by the International Programme on Chemical Safety, established within the World Health Organization with the cooperation of the United Nations Environment Programme and the International Labour Organisation, and the Scientific Committee on Problems of the Environment, itself a body of the International Council of Scientific Unions. The goal of the 13th meeting of SGOMSEC (26-31 January 1997) was to assess the status of alternative methodologies for the safety assessment of chemical and physical agents.

- ✓ Additional relevant publications suggested by Workshop participants.

- ✓ Draft White Paper "The National Center for Toxicogenomics," September 5, 2000

The additional publications to consider are organized according to the appropriate section (e.g., General Strategies, Breakout Groups 1, 2, and 3) of the **Background Document** located in **Section E** of the **Background Materials And Supplemental Information Workbook**. To assist the Workshop participant, publications previously listed in the “Publications Containing Further Information” subsections of the

Background Document are included. For ease of identification, these publications are italicized. Each relevant SCOMSEC publication is listed, and a copy of its abstract provided. Additional relevant publications to consider, as suggested by Workshop participants, are bolded. A list of publications cited within the Background Document can be found in that Document (see Section E of the Workbook).

Section 3.0 *In Vitro* Test Methods for Predicting *In Vivo* Toxicity—General Strategies

Balls, M. 1998. Commentary: Mechanistic approaches and the development of alternative toxicity test methods. Environ. Hlth Perspect. 106 (Supple. 2):453-458.

Abstract. A mechanism can be defined as an explanation of an observed phenomenon that explains the processes underlying the phenomenon in terms of events at lower levels of organization. A prerequisite for new, more mechanistic, approaches, which would use in vitro systems rather than conventional animal analogy models, is a strengthening of the underlying scientific basis of toxicity testing. This will require greater recognition of the differences between fidelity and discrimination models and between analogy and correlation models. The development of high-fidelity, high-discrimination tests with a sound mechanistic basis will also require greater appreciation of the interdependence of all the components of test systems and the development of new alternative (i.e., nonanimal) testing strategies that can provide the specific knowledge needed for making relevant and reliable predictions about the potential effects of chemicals and

products in human beings. The optimal use of this new knowledge will require fundamental changes to current practices in risk assessment. -- Environ Health Perspect 106(Suppl 2):453-457 (1998).
http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-2/453-457balls/abstract.html

Balls, M., B.J. Blaauboer, J.H. Fentem, L. Bruner, R.D. Combes, B. Ekwall, R.J. Fielder, A. Guillouzo, R.W. Lewis, D.P. Lovell, C.A. Reinhardt, G. Repetto, D. Sladowski, H. Spielmann, and F. Zucco. 1995. Practical aspects of the validation of toxicity test procedures –The report and recommendations of ECVAM Workshop 5. ATLA 23:129-147.

Bruner, L.H., G.J. Carr, R.D. Curren, and M. Chamberlain. 1998. Validation of alternative methods for toxicity testing. Environ. Hlth Perspect. 106 (Supple. 2):477-484.

Abstract. Before nonanimal toxicity tests may be officially accepted by regulatory agencies, it is generally agreed that the validity of the new methods must be demonstrated in an independent, scientifically sound validation program. Validation has been defined as the demonstration of the reliability and relevance of a test method for a particular purpose. This paper provides a brief review of the development of the theoretical aspects of the validation process and updates current thinking about objectively testing the performance of an alternative method in a validation study. Validation of alternative methods for eye irritation testing is a specific example illustrating important concepts. Although discussion focuses on the validation of alternative methods intended to replace current in vivo toxicity tests, the procedures can be used to assess the performance of alternative methods intended for other uses. -- Environ Health Perspect 106(Suppl 2):477-484 (1998).
http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-2/477-484bruner/abstract.html

Curren, R., L. Bruner, A. Goldberg, and E. Walum. 1998. Validation and acute toxicity testing. Environ. Hlth Perspect. 106 (Supple. 2):419-426.

Abstract: Scientific principles demand that before newly developed alternative methods for safety testing are fully embraced by the industrial or regulatory community, they reliably and reproducibly predict the designated toxic end point. The process used to determine reliability and reproducibility is termed validation, and it generally culminates with a highly controlled, blinded study using multiple chemicals and laboratories. It is imperative that the validation study is designed to confirm the previously established reproducibility and predictive power of the assay. Much has been learned recently about the practical aspects of validation through investigation of alternative methods for acute toxicity testing, i.e., those methods that assess acute systemic toxicity, skin irritation, and eye irritation. Although considerable progress has been made--many alternative tests are now commonly used in various industrial settings--there have been few tests that have successfully passed a complete validation. Some of the barriers to successful validation have been a) lack of high-quality, reproducible animal data; b) insufficient knowledge of the fundamental biologic processes involved in acute toxicity; and c) the development of truly robust in vitro assays that can accurately respond to materials with a wide range of chemical and physical characteristics. It is recommended that to progress in the areas of eye and skin irritation we need to expand our knowledge of toxic markers in humans and the biochemical basis of irritation; progress in the area of acute systemic toxicity will require the development of in vitro models to determine gastrointestinal uptake, blood-brain barrier passage, and biotransformation. -- Environ Health Perspect 106(Suppl 2):419-425 (1998).

Seibert, H., M. Gülden, and J.-U. Voss. 1994. An in vitro toxicity testing strategy for the classification and labelling of chemicals according to their potential acute lethal potency. Toxicol. In Vitro 8:847-850.

Seibert, H., M. Gülden, and J.-U. Voss. 1994. An in vitro toxicity testing strategy for the classification and labelling of chemicals according to their potential acute lethal potency. Toxicol. In Vitro 8:847-850.

Stokes, W.S., and E. Marafante. 1998. Introduction and summary of the 13th meeting of the Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC): Alternative testing methodologies. Environ. Hlth Perspect. 106 (Supple. 2):405-412.

Abstract. A workshop on alternative toxicological testing methodologies was convened by the Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC) 26-31 January 1997 in Ispra, Italy, at the European Centre for the Validation of Alternative Methods. The purpose of the workshop was to assess the current status of alternative testing methodologies available to evaluate adverse human health and environmental effects of chemicals. Another objective of the workshop was to identify and recommend research needed to fill knowledge gaps that would lead to new test methodologies. Four work groups were established to address conceptual issues, acute toxicity, organ toxicity, and ecotoxicology. A joint workshop report was prepared for each topic and included recommendations for the development and use of alternative methods. Participants concluded that alternative methods and approaches are available that can be incorporated into tiered strategies for toxicological assessments. Use of these methods will reduce the numbers of animals required, and in some instances reduce animal pain and distress. It was recommended that future efforts to develop test methods should emphasize mechanism-based methods that can provide improved predictions of toxicity. Continued international cooperation was encouraged to facilitate future progress in the development of alternative toxicological testing methods. These methods will provide for improvements in human health protection, environmental protection, and animal welfare. -- Environ Health Perspect 106(Suppl 2):405-412

(1998). http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-2/405-412stokes/abstract.html

Walum, E., Acute oral toxicity. Environ. Hlth Perspect. 106 (Supple. 2):497-504.

Abstract. The purposes of acute toxicity testing are to obtain information on the biologic activity of a chemical and gain insight into its mechanism of action. The information on acute systemic toxicity generated by the test is used in hazard identification and risk management in the context of production, handling, and use of chemicals. The LD50 value, defined as the statistically derived dose that, when administered in an acute toxicity test, is expected to cause death in 50% of the treated animals in a given period, is currently the basis for toxicologic classification of chemicals. For a classical LD50 study, laboratory mice and rats are the species typically selected. Often both sexes must be used for regulatory purposes. When oral administration is combined with parenteral, information on the bioavailability of the tested compound is obtained. The result of the extensive discussions on the significance of the LD50 value and the concomitant development of alternative procedures is that authorities today do not usually demand classical LD50 tests involving a large number of animals. The limit test, the fixed-dose procedure, the toxic class method, and the up-and-down methods all represent simplified alternatives using only a few animals. Efforts have also been made to develop in vitro systems; e.g., it has been suggested that acute systemic toxicity can be broken down into a number of biokinetic, cellular, and molecular elements, each of which can be identified and quantified in appropriate models. The various elements may then be used in different combinations to model large numbers of toxic events to predict hazard and classify compounds. -- Environ Health Perspect 106(Suppl 2):497-503 (1998). http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-2/497-503walum/abstract.html

Walum, E., M. Balls, B. Bianchi, B. Blaauboer, G. Bolcsfoldi, A. Guillouzo, G.A. Moor, L. Odland, C.A. Reinhardt, and H. Spielmann. 1992. ECITTS: An integrated approach for the application of in vitro tests systems for the hazard assessment of chemicals. ATLA 20:406-428.

Section 3.1 Quantitative Structure Activity Relationship (QSAR) Methods

Barratt, M.D. 1998. Integration of QSAR and in vitro toxicology. Environ. Hlth Perspect. 106 (Supple. 2):459-466.

Abstract. The principles of quantitative structure-activity relationships (QSAR) are based on the premise that the properties of a chemical are implicit in its molecular structure. Therefore, if a mechanistic hypothesis can be proposed linking a group of related chemicals with a particular toxic end point, the hypothesis can be used to define relevant parameters to establish a QSAR. Ways in which QSAR and in vitro toxicology can complement each other in development of alternatives to live animal experiments are described and illustrated by examples from acute toxicological end points. Integration of QSAR and in vitro methods is examined in the context of assessing mechanistic competence and improving the design of in vitro assays and the development of prediction models. The nature of biological variability is explored together with its implications for the selection of sets of chemicals for test development, optimization, and validation. Methods are described to support the use of data from in vivo tests that do not meet today's stringent requirements of acceptability. Integration of QSAR and in vitro methods into strategic approaches for the replacement, reduction, and refinement of the use of animals is described with examples. -- Environ Health Perspect 106(Suppl 2):459-465 (1998). http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-2/459-465barratt/abstract.html

Dearden, J.C., M.D. Barratt, R. Benigni, D.W. Briston, R.D. Combes, M.T.D. Cronin, P.N. Judson, M.P. Payne, A.M. Richard, M. Tichy, A.P. Worth and J.J. Yourick. 1997.

The development and validation of expert systems for predicting toxicity. ATLA 25:223-252.

Free, S.M., and J.W. Wilson. 1964. A mathematical contribution to structure-activity studies. J. Med. Chem. 7:395-399.

Hansch, C., and T. Fujita. 1964. ρ, σ, π Analysis. A method for the correlation of biological activity and chemical structure. J. Am. Chem. Soc. 86:1616-1626.

Section 4.0 *In Vitro* Methods for Assessing Acute Toxicity (Breakout Group I) (MEIC-specific publications are not included-see Section 4.1.4 of the Background Document (Section I of the Workbook).

Clothier, R.H., L.M. Hulme, M. Smith and M. Balls. 1987. Comparison of the *in vitro* cytotoxicities and acute *in vivo* toxicities of 59 chemicals. Molecular Toxicology 1:571-577.

Halle, W., and H. Spielmann. 1992. Two procedures for the prediction of acute toxicity (LD_{50}) from cytotoxicity data. ATLA 20:40-49.

Seibert, H., M. Gülden, and J.-U. Voss. 1994. An in vitro toxicity testing strategy for the classification and labelling of chemicals according to their potential acute lethal potency. Toxicol. In Vitro 8:847-850.

Seibert, H., M. Gülden, and J.-U. Voss. 1994. An in vitro toxicity testing strategy for the classification and labelling of chemicals according to their potential acute lethal potency. Toxicol. In Vitro 8:847-850.

Section 5.0 *In Vitro* Methods for Assessing Acute Toxicity—Toxicokinetic Determinations (Breakout Group 2)

Blaauboer B.J., and J. DeJongh. 1998. An integrated approach to the prediction of systemic toxicity using computer-based biokinetic models and biological in vitro test methods. Report for the Dutch Platform Alternatives to Animal Testing (PAD), The Hague, The Netherlands. 34 pp.

Blaauboer, B.J., M. Balls, M. Barratt, S. Casati, S. Coecke, M.K. Mohamed, J. Moore, D. Rall, K.R. Smith, R. Tennant, B.A. Schwetz, W.S. Stokes, and M. Younes. 1998. Alternative testing methodologies and conceptual issues. Environ. Hlth Perspect. 106 (Supple. 2):413-418.

Abstract: Substantial world-wide resources are being committed to develop improved toxicological testing methods that will contribute to better protection of human health and the environment. The development of new methods is intrinsically driven by new knowledge emanating from fundamental research in toxicology, carcinogenesis, molecular biology, biochemistry, computer sciences, and a host of other disciplines. Critical evaluations and strong scientific consensus are essential to facilitate adoption of alternative methods for use in the safety assessment of drugs, chemicals, and other environmental factors. Recommendations to hasten the development of new alternative methods included increasing emphasis on the development of mechanism-based methods, increasing fundamental toxicological research, increasing training on the use of alternative methods, integrating accepted alternative methods into toxicity assessment, internationally harmonizating chemical toxicity classification schemes, and increasing international cooperation to develop, validate, and gain acceptance of alternative methods. -- Environ Health Perspect 106(Suppl 2):413-418 (1998).
http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-2/413-418blaauboer/abstract.html

Blaauboer B.J., M.D. Barratt, and J.B. Houston. 1999. The integrated use of alternative methods in toxicological risk evaluation. ECVAM Integrated Testing Strategies Task Force Report 1. Alternatives to Laboratory Animals 27: 229-237.

Blaauboer, B.J., M.K. Bayliss, J.V. Castell, C.T.A. Evelo, J.M. Frazier, K. Groen, M. Guelden, A. Guillouzo, A.M. Hissink, J.B. Houston, G. Johanson, J. de Jongh, G.L. Kedderis, C.A. Reinhardt, J.J.M. Van de Sandt and G. Semino. 1996. The use of biokinetics and *in vitro* methods in toxicological risk evaluation. ATLA 24:473-497.

Blaauboer, B.J., A.R. Boobis, J.V. Castell, S. Coecke, G.MM. Groothuis, A. Guillouzo, T.J. Hall, G.M. Hawksworth, G. Lorenzen, H.G. Miltenburger, V. Rogiers, P. Skett, P. Villa, and F.J Wiebel. 1994. The practical applicability of hepatocyte cultures in routine testing. The report and recommendations of ECVAM Workshop 1. ATLA 22:231-241.

Blaauboer B.J., A. Forsby, J.B. Houston, M. Beckman, R.D. Combes, and J. DeJongh. 2000. An integrated approach to the prediction of systemic toxicity using biokinetic models and biological in vitro test methods. In: Progress in the Reduction Refinement and Replacement of Animal Experimentation. (Balls M, van Zeller A-M and Halder ME, eds). Elsevier, Amsterdam, in press.

DeJongh J., A. Forsby, J.B. Houston, M. Beckman, R. Combes, B.J. Blaauboer. 1999. An integrated approach to the prediction of systemic toxicity using computer-based biokinetic models and biological in vitro test methods: overview of a prevalidation study based on the ECITTS project. Toxicology in Vitro 13:549-554.

DeJongh, J., M. Nordin-Andersson, B.A. Ploeger B.A. and A. Forsby. 1999. Estimation of systemic toxicity of acrylamide by integration of in vitro toxicity data with kinetic simulations. Toxicology Appl. Pharmac. 158(3):261-268.

DeJongh, J., H.J.M. Verhaar, J.L.M. Hermens. 1997. A quantitative property-property relationship (QPPR) approach to estimate *in vitro* tissue-blood partition coefficients of organic chemicals in rats and humans. Arch. Toxicol. 72:17-25.

Ericsson, A.C., and E. Walum. 1988. Differential effects of allyl alcohol on hepatocytes and fibroblasts demonstrated in roller chamber co-cultures. ATLA 15:208-213.

Guillouzo, A. 1998. Liver cell models in in vitro toxicology. Environ. Hlth Perspect. 106 (Supple. 2):511-532.

Abstract. In vitro liver preparations are increasingly used for the study of hepatotoxicity of chemicals. In recent years their actual advantages and limitations have been better defined. The cell models, slices, and mainly primary hepatocyte cultures, appear to be the most powerful in vitro systems, as liver-specific functions and responsiveness to inducers are retained either for a few days or several weeks depending on culture conditions. Maintenance of phase I and phase II xenobiotic metabolizing enzyme activities allows various chemical investigations to be performed, including determination of kinetic parameters, metabolic profile, interspecies comparison, inhibition and induction effects, and drug-drug interactions. In vitro liver cell models also have various applications in toxicology: screening of cytotoxic and genotoxic compounds, evaluation of chemoprotective agents, and determination of characteristic liver lesions and associated biochemical mechanisms induced by toxic compounds. Extrapolation of the results to the in vivo situation remains a matter of debate. Presently, the most convincing applications of liver cell models are the studies on different aspects of metabolism and mechanisms of toxicity. For the future, there is a need for better culture conditions and differentiated hepatocyte cell lines to overcome the limited availability of human liver tissues. In addition, strategies for in vitro analysis of potentially toxic chemicals

must be better defined. -- Environ Health Perspect 106(Suppl 2):511-532 (1998). http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-2/511-532guillouzo/abstract.html

Paillard, F., F. Finot, I. Mouche, A. Prenez, and J. A. Vericat. 1999. Use of primary cultures of rat hepatocytes to predict toxicity in the early development of new chemical entities. Toxicol. In Vitro 13:693-700.

Voss, J.-U., and H. Seibert. 1991. Microcarrier-attached rat hepatocytes as a xenobiotic-metabolising system in co-cultures. Cell Biol. Toxicol. 7:387-399.

Voss, J.-U., and H. Seibert. 1992. Toxicity of glycols and allyl alcohol evaluated by means of co-cultures of microcarrier-attached rat hepatocytes and Balb/c 3T3 mouse fibroblasts. ATLA 20:266-270.

Section 6.0 *In Vitro* Methods for Assessing Acute Toxicity—Specific Organ Toxicity and Mechanisms (Breakout Group 3)

Atterwill, C.K., A. Bruinink, J. Drejer, E. Duarte, E.M. Abdulla, C. Meredith, P. Nicotera, C. Regan, E. Rodriguez-Farre, M.G. Simpson, R. Smith, B. Veronesi, H. Vijverberg, E. Walum and D.C. Williams. 1994. *In vitro* neurotoxicity testing. ATLA 22:350-362.

Bach, P.H., A.E.M. Vickers, R.Fisher, A. Baumann, E. Brittebo, D.J. Carlile, H.J. Koster, B.G. Lake, F. Salmon, T.W. Sawyer and G. Skibinski. 1996. The use of tissue slices for pharmacotoxicology studies. ATLA 24:893-923.

Costa, L.G. 1998. Neurotoxicity testing: A discussion of in vitro alternatives. Environ. Hlth Perspect. 106 (Supple. 2):505-510.

Abstract. A large number of chemicals may exert adverse effects on the central and/or peripheral nervous system. A commonly recommended strategy for neurotoxicity testing is that of a tiered approach aimed at identifying and characterizing the neurotoxicity of a compound. Guidelines exist in the United States and other countries that define the tests to be utilized in tier 1 testing. To address problems related to the increasing cost and time required for toxicity testing, the increasing number of chemicals being developed, and the concern of animal welfare activists, attention is currently being devoted to in vitro alternatives. This paper addresses the use of in vitro systems in neurotoxicology, and their potential role in a general strategy for neurotoxicity testing. The advantages and disadvantages of in vitro approaches for mechanistic studies and for screening of neurotoxicants are discussed. Suggestions for further validation studies are proposed. -- Environ Health Perspect 106(Suppl 2):505-510 (1998). http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-2/505-510costa/abstract.html

Ekwall, B., C. Clemedson, Ba. Ekwall, P. Ring, and L. Romert. 1999. EDIT: A New International Multicentre Programme to Develop and Evaluate Batteries of In Vitro Tests for Acute and Chronic Systemic Toxicity. ATLA 27:339-349.

Forsby, A., F. Pilli, V. Bianchi, and E. Walum. 1995. Determination of critical cellular neurotoxic concentrations in human neuroblastoma (SH-SY5Y) cell cultures. ATLA 23:800-811.

Guillouzo, A. 1998. Liver cell models in in vitro toxicology. Environ. Hlth Perspect. 106 (Supple. 2):511-532.

Abstract. In vitro liver preparations are increasingly used for the study of hepatotoxicity of chemicals. In recent years their actual advantages and limitations have been better defined. The cell models, slices, and mainly primary hepatocyte cultures, appear to be the most powerful in vitro systems, as liver-specific functions

and responsiveness to inducers are retained either for a few days or several weeks depending on culture conditions. Maintenance of phase I and phase II xenobiotic metabolizing enzyme activities allows various chemical investigations to be performed, including determination of kinetic parameters, metabolic profile, interspecies comparison, inhibition and induction effects, and drug-drug interactions. In vitro liver cell models also have various applications in toxicology: screening of cytotoxic and genotoxic compounds, evaluation of chemoprotective agents, and determination of characteristic liver lesions and associated biochemical mechanisms induced by toxic compounds. Extrapolation of the results to the in vivo situation remains a matter of debate. Presently, the most convincing applications of liver cell models are the studies on different aspects of metabolism and mechanisms of toxicity. For the future, there is a need for better culture conditions and differentiated hepatocyte cell lines to overcome the limited availability of human liver tissues. In addition, strategies for in vitro analysis of potentially toxic chemicals must be better defined. -- Environ Health Perspect 106(Suppl 2):511-532 (1998). http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-2/511-532guillouzo/abstract.html

Gülden, M., H. Seibert, and J.-U. Voss. 1994. Inclusion of physicochemical data in quantitative comparisons of in vitro and in vivo toxic potencies. ATLA 22:185-192.

Gülden, M., H. Seibert, and J.-U. Voss. 1994. The use of cultured skeletal muscle cells in testing for acute systemic toxicity. Toxicology In Vitro 8:779-782.

Halle, W., and H. Spielmann. 1992. Two procedures for the prediction of acute toxicity (LD_{50}) from cytotoxicity data. ATLA 20:40-49.

Hawksworth, G.M., P.H. Back, J.F. Nagelkerke, W. Dekant, J.E. Diezi, E. Harpur, E.A. Lock, C. MacDonals, J.-P. Morin, W. Pfaller, F.A.J.J.L. Rutten, M.P. Ryan, H.J. Toutain, and A. Tevisan. 1995. Nephrotoxicity testing *in vitro*. ATLA 23:713-727.

Karol, M.H. 1998. Target organs and systems: Methodologies to assess immune system function. Environ. Hlth Perspect. 106 (Supple. 2):533-540.

Abstract. Immunotoxicity encompasses both reduced and heightened immune function. Diverse chemicals can impair functioning of the immune system. Both monographs and books have been devoted to detailed descriptions of immunotoxicity. This paper gives a brief overview of the methods currently used to assess the immunotoxic potential of chemicals. It also discusses the trend toward the use of alternative methods to mammalian models, such as feral species, in vitro assays, and computational models. The strategy of using a tier approach to screen chemicals for immunotoxicity is described, together with the rationale for, and limitations of, this approach. Interpretation of data with regard to clinical disease and human health is addressed. The immune system poses substantial complexities in this regard as the system has functional reserve and functional redundancy. -- Environ Health Perspect 106(Suppl 2):533-540 (1998).
http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-2/533-540karol/abstract.html

Norden-Andersson, M., A. Forsby, N. Heldring, J. DeJongh, P. Kjellstrand and E. Walum. 1998. Neurite degeneration in differentiated human neuroblastoma cells. Toxicology *in Vitro* 12:557-560.

Parchment, R.E. 1998. Alternative testing systems for evaluating noncarcinogenic, hematologic toxicity. Environ. Hlth Perspect. 106 (Supple. 2):541-558.

Abstract. Hematopoietic tissues are the targets of numerous xenobiotics. Clinical hematotoxicity is either a decrease or an increase in peripheral blood cell counts in one or more cell lineages--a cytopenia or a cytosis, respectively--that carries a risk of an adverse clinical event. The purpose of in vitro hematotoxicology is the prediction

of these adverse hematologic effects from the effects of the toxicants on human hematopoietic targets under controlled experimental conditions in the laboratory. Building on its important foundations in experimental hematology and the wealth of hematotoxicology data found in experimental oncology, this field of alternative toxicology has developed rapidly during the past decade. Although the colony-forming unit-granulocyte / monocyte neutrophil progenitor is most frequently evaluated, other defined progenitors and stem cells as well as cell types found in the marrow stroma can be evaluated in vitro . End points have been proposed for predicting toxicant exposure levels at the maximum tolerated dose and the no observable adverse effect level for the neutrophil lineage, and several clinical prediction models for neutropenia have developed to the point that they are ready for prospective evaluation and validation in both preclinical species and humans. Known predictive end points are the key to successful comparisons across species or across chemical structures when in vitro dose-response curves are nonparallel. Analytical chemistry support is critical for accurate interpretation of in vitro data and for relating the in vitro pharmacodynamics to the in vivo pharmacokinetics. In contrast to acute neutropenia, anemia and acute thrombocytopenia, as well as adverse effects from chronic toxicant exposure, are much more difficult to predict from in vitro data. Pharmacologic principles critical for clinical predictions from in vitro data very likely will apply to toxicities to other proliferative tissues, such as mucositis. -- Environ Health Perspect 106(Suppl 2):541-557 (1998). http: / / ehpnet1.niehs.nih.gov / docs / 1998 / Suppl-2 / 541-557parchment / abstract.html

Pfaller, W., and G. Gtraunthaler. 1998. Nephrotoxicity testing in vitro --what we know and what we need to know. Environ. Hlth Perspect. 106 (Supple. 2):559-570.

Abstract. The kidney is affected by many chemicals. Some of the chemicals may even contribute to end-stage renal disease and thus contribute considerably to health care costs. Because of the large functional reserve of the kidney, which masks signs

of dysfunction, early diagnosis of renal disease is often difficult. Although numerous studies aimed at understanding the mechanisms underlying chemicals and drugs that target various renal cell types have delivered enough understanding for a reasonable risk assessment, there is still an urgent need to better understand the mechanisms leading to renal cell injury and organ dysfunction. The increasing use of in vitro techniques using isolated renal cells, nephron fragments, or cell cultures derived from specific renal cell types has improved our insight into the molecular mechanisms involved in nephrotoxicity. A short overview is given on the various in vitro systems currently used to clarify mechanistic aspects leading to sublethal or lethal injury of the functionally most important nephron epithelial cells derived from various species. Whereas freshly isolated cells and nephron fragments appear to represent a sufficient basis to study acute effects (hours) of nephrotoxins, e.g., on cell metabolism, primary cultures of these cells are more appropriate to study long-term effects. In contrast to isolated cells and fragments, however, primary cultures tend to first lose several of their in vivo metabolic properties during culture, and second to have only a limited life span (days to weeks). Moreover, establishing such primary cultures is a time-consuming and laborious procedure. For that reason many studies have been carried out on renal cell lines, which are easy to cultivate in large quantities and which have an unlimited life span. Unfortunately, none of the lines display a state of differentiation comparable to that of freshly isolated cells or their primary cultures. Most often they lack expression of key functions (e.g., gluconeogenesis or organic anion transport) of their in vivo correspondents. Therefore, the use of cell lines for assessment of nephrotoxic mechanisms will be limited to those functions the lines express. Upcoming molecular biology approaches such as the transduction of immortalizing genes into primary cultures and the utilization of cells from transgenic animals may in the near future result in the availability of highly differentiated renal cells with markedly extended life spans and near in vivo characteristics that may facilitate the use of renal cell culture for routine screening of nephrotoxins. -- Environ Health Perspect 106(Suppl 2):559-569

(1998). http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-2/559-569pfaller/abstract.html

Spielmann, H., N.P. Bochkov, L. Costa, L. Gribaldo, A. Guillouzo, J.J. Heindel, M. Karol, R. Parchment, W. Pfaller, P.P. Peraita, and T. Zacharewski. 1998. Alternative testing methodologies for organ toxicity. Environ. Hlth Perspect. 106 (Supple. 2):427-440.

Abstract. In the past decade in vitro tests have been developed that represent a range of anatomic structure from perfused whole organs to subcellular fractions. To assess the use of in vitro tests for toxicity testing, we describe and evaluate the current status of organotypic cultures for the major target organs of toxic agents. This includes liver, kidney, neural tissue, the hematopoietic system, the immune system, reproductive organs, and the endocrine system. The second part of this report reviews the application of in vitro culture systems to organ specific toxicity and evaluates the application of these systems both in industry for safety assessment and in government for regulatory purposes. Members of the working group (WG) felt that access to high-quality human material is essential for better use of in vitro organ and tissue cultures in the risk assessment process. Therefore, research should focus on improving culture techniques that will allow better preservation of human material. The WG felt that it is also important to develop and make available relevant reference compounds for toxicity assessment in each organ system, to organize and make available via the Internet complete in vivo toxicity data, including human data, containing dose, end points, and toxicokinetics. The WG also recommended that research should be supported to identify and to validate biological end points for target organ toxicity to be used in alternative toxicity testing strategies. -- Environ Health Perspect 106(Suppl 2):427-439(1998). http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-2/427-439spielmann/abstract.html.

Walum, E., M. Balls, B. Bianchi, B. Blaauboer, G. Bolcsfoldi, A. Guillouzo, G.A. Moor, L. Odland, C.A. Reinhardt, and H. Spielmann. 1992. ECITTS: An integrated approach for the application of in vitro tests systems for the hazard assessment of chemicals. ATLA 20:406-428.