Appendix

Annotated Reading List

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This appendix contains a critically reviewed list of works related to in vitro neurotoxicology. The list has been carefully selected and annotated by the contributors to include reference books on neurotoxicology, books and essays on in vitro neurotoxicology, books and chapters on related methods, and important review articles that have appeared in the past 10 yr.

BOOKS ON NEUROTOXICOLOGY

Aschner, M. and Kimelberg, H. K. (eds.) (1996) *The Role of Glia in Neurotoxicity*, CRC, New York

This is the first book to focus exclusively on the roles of neuroglia in neurotoxicity. Contributors review and explore potential sites for neurotoxic action in glial–neuronal interactions in both the central and peripheral nervous systems. Individual chapters address methodologies and concepts of neurotoxicology, including many examples of in vitro approaches. This publication was intended to fill a gap in the literature. With the increasing importance of glia, a journal, *Glia*, has been dedicated to studies on these cells. In addition, many recent textbooks have included discussion of glia, encompassing neurophysiology, neuroanatomy, neuroscience, neurochemistry, and neuropharmacology. None, however, included the role of glia in neurotoxicity, a timely topic and a subject in its own right.

Chang, L.W. (ed.) (1994) *Principles of Neurotoxicology*, Marcel Dekker, New York

This standard reference provides a comprehensive overview of principles and modern concepts of neurotoxicology. The work contains major sections on the central nervous system, behavioral neurotoxicology, biochemical and molecular neurotoxicology, and developmental neurotoxicology.

From: *Methods in Pharmacology and Toxicology: In Vitro Neurotoxicology: Principles and Challenges*
Edited by: E. Tiffany-Castiglioni © Humana Press Inc., Totowa, NJ

Costa, L. and Manzo, L. (eds.) (1998) *Occupational Neurotoxicology*, CRC, New York

This book provides a concise overview of important concerns of the relatively new specialty of occupational neurotoxicology. Among them are commonly encountered workplace neurotoxicants, signs and symptoms of neurotoxicity, detection and monitoring of human exposure by biomarkers, epidemiology, and diagnostic methods.

Harry, G. J. (ed.) (1994) *Developmental Neurotoxicology*, CRC, New York

This book examines the biological characteristics of the developing nervous system that increase its vulnerability to damage by exposure to environmental toxicants. Contributing authors discuss functional alterations that occur at exposure levels too low to produce structural teratogenesis.

Massaro, E. J. (ed.) (2002) *Handbook of Neurotoxicology, Volumes I and II*, Humana, Totowa, NJ

This two-volume set provides a current overview of important topics in contemporary neurotoxicology. In Volume I, 28 topics are covered under 4 sections: pesticides, metals, microbial toxins, and animal toxins (venoms). In Volume II, 21 topics are covered under 4 sections: developmental neurotoxicology, drugs of abuse, imaging, and neurobehavioral assessment methods. Section editors and chapter contributors are international experts from academia, industry, and government agencies. Volume I, in particular, offers excellent further reading on topics relevant to in vitro neurotoxicology, such as pesticide effects on ion channels, mechanisms of lead neuronal toxicity, interactions of metals with the zinc-finger motif, and the blood–brain barrier in metal toxicity.

Slikker, W. B. and Chang, L. W. (eds.) (1998) *Handbook of Developmental Neurotoxicology*, Academic Press, New York

This highly comprehensive multidisciplinary reference addresses the mechanisms and relevance of the developmental toxicity of chemicals. The subject is divided into seven major sections on cellular and molecular morphogenesis, developmental biology and toxicology, synaptogenesis and neurotransmission, nutrient and chemical disposition, behavioral assessment, clinical assessment and epidemiology, specific neurotoxic syndromes, and risk assessment.

Tilson, H. A. and Harry, G. J. (1999) *Neurotoxicology*, 2nd ed., Target Organ Toxicology Series, Taylor & Francis, Philadelphia

The major focuses of this edition are the discovery of sites and mechanisms of neurotoxicity and their value in improving risk assessment. The text focuses primarily on the neurobiological basis underlying neurotoxic sites and modes of action. Contributing authors provide 15 chapters on topics spanning molecular biological approaches in neurotoxicology, in vitro neurotoxicology, specific cellular and biochemical processes damaged by toxicants, effects on learning and behavior, and emerging concepts in risk assessment.

BOOKS AND POSITION PAPERS ON IN VITRO NEUROTOXICOLOGY

Aschner, M., Allen, J. W., Kimelberg, H. K., LoPachin, R. M., and Streit, W. J. (1999) Glial cells in neurotoxicity development. *Annu. Rev. Pharmacol. Toxicol.* **39,** 151–173

Experts on each of the major classes of neuroglia (astrocytes, oligodendrocytes, microglia, and Schwann cells) present models for neurotoxic sites of action that involve glia. Glial interactions with neurons and other glia that underlie nervous system development and function are examined. The work described is based on in vitro and in vivo models.

Aschner, M. and Kerper, L. E. (2000) Transport of metals in the nervous system, in *Molecular Biology and Toxicology of Metals* (Koropatnick, D.J. and Zalups, R. K., eds.), Taylor & Francis, London, pp. 276–299

The blood–brain barrier (BBB) is a specialized structure responsible for the maintenance of the neuronal microenvironment. A pivotal function of the endothelial cells comprising the blood–brain barrier is to regulate the selective transport and metabolism of substances from blood to brain, as well as their transport in the opposite direction. This chapter addresses the development of the blood–brain barrier, with emphasis on the crosstalk between astrocytes and endothelial cells, as well as known mechanisms of metal transport by endothelial cells.

Audesirk, G. J. (1997) *In vitro* systems in neurotoxicological studies, in *Nervous System and Behavioral Toxicology, Comprehensive Toxicology, Volume 11* (Lowndes, H. E. and Reuhl, K. R., eds.), Elsevier Science, Amsterdam, pp. 431–446

This chapter focuses on complementarity between in vitro and in vivo approaches. A concise overview of in vitro systems is provided. Of special interest are discussions of acute versus semichronic neurotoxicity and the problem of concentration and duration of exposure in vitro.

Costa, L. G. (1998) Neurotoxicity testing: a discussion of in vitro alternatives. *Environ. Health Perspect.* **106(Suppl.),** 505–510.

In addition to briefly discussing the advantages and disadvantages of in vitro systems, the author thoughtfully discusses in vitro systems for mechanistic studies and neurotoxicity screening. Tiered approaches are suggested, because no single invitro system can reliably detect all possible end points.

Costa, L. G. (1998) Biochemical and molecular neurotoxicology: relevance to biomarker development, neurotoxicity testing and risk assessment, *Toxicol. Lett.* **102–103,** 417–421

Biomarkers are generally divided into three categories: biomarkers of exposure, effect, and susceptibility. This commentary addresses biomarkers of effect and their cross-disciplinary use in animal toxicity studies, epidemiology, and in vitro toxicity testing. The example of organophosphorus insecticide neurotoxicity is explored.

Deng, W. and Poretz, R. D. (2003) Oligodendroglia in developmental neurotoxicity. *Neurotoxicology* **24,** 161–178

This is the first contemporary review to address the roles of oligodendroglia in developmental neurotoxicity. Topics covered are the developmental lineage of oligodendrocytes, maturational characteristics in vivo and in vitro, and modulation of differentiation in cell culture models. The well-defined oligodendrocyte lineage is presented as an advantageous system for investigations of developmental neurotoxicity. Recent work from the authors' laboratory on lead neurotoxicity is reviewed.

Ehrich, M. and Veronesi, B. (1998) In vitro neurotoxicology, in *Neurotoxicology* (Tilson, H. A. and Harry, G. J., eds.), Taylor & Francis, Philadelphia, pp. 37–50

This chapter provides a general review of methods and examples of their use for neurotoxicology. The biological basis underlying neurotoxic sites and modes of action is addressed.

Harry, G. J., Billingsley, M., Bruinink, A., Campbell, I. L., Classen, W., Dorman, D. C., Galli, C., Ray, D., Smith, R. A., and Tilson, H. A. (1998) In vitro techniques for the assessment of neurotoxicity. *Environ. Health Perspect.* **106(Suppl.),** 131–158

This work is an extensive review and discussion of the topic prepared as a document for the International Program on Chemical Safety (IPCS) and cosponsored by the United Nations Environment Program, World Health Organization, and International Labor Organization. The focus of this review is the usefulness of in vitro techniques for the identification of neurotoxic hazards. End points receive particular attention because of their use in distinguishing between a pharmacologic and neurotoxic response. This work is also valuable as an introductory resource for the reader new to culture techniques, as several common techniques, cell lines, and problems encountered in culture are discussed.

Harry, G. J. and Tilson, H. A. (ed.) (1999) *Neurodegeneration Methods and Protocols*, Humana, Totowa, NJ

The objective of this book is to develop an understanding of and technical ability in various cellular and molecular techniques for studying many aspects of nervous system cell biology. The protocols in this book span a multidisciplinary range of cellular and molecular approaches and should allow investigators to address research questions directed toward understanding nervous system function, injury, degeneration, and the repair/regenerative process.

Pentreath, V. W. (ed.) (1999) *Neurotoxicology In Vitro*, Taylor & Francis, Philadelphia

This excellent work is suggested as a companion volume to the current book. The book contains concise reviews of principles of neurobiology, commonly used cell lines, and selected in vitro techniques. The uses of in vitro methods for mechanisms versus screening studies are also thoughtfully addressed.

Philbert, M. A. and Aschner, M. (1997) Glial cells, in *Nervous System and Behavioral Toxicology, Comprehensive Toxicology, Volume 11* (Lowndes, H. E. and Reuhl, K. R., eds.), Elsevier Science, Amsterdam, pp. 217–236

The dynamic role of glia in the maintenance of normal neural tissues and their potential involvement in degenerative disease processes and following exposure to xenobiotics are discussed. This review provides a general overview of glia as targets and mediators of neurotoxicity in the nervous system.

Tiffany-Castiglioni, E. and Qian, Y. (2001) Astroglia as metal depots: molecular mechanisms for metal accumulation, storage and release. *Neurotoxicology* **22,** 577–592

This review extends the lead-sink hypothesis for astroglia to other metals. In vivo and in vitro evidence that mercury, manganese, and copper might be selectively accumulated by astroglia is examined.

Tiffany-Castiglioni, E., Ehrich, M., Dees, W. L, Costa, L.G., Kodavanti, P. R. S., Lasley, S. M., Oortgiesen, M., and Durham, H. D. (1999) Bridging the gap between in vitro and in vivo models for neurotoxicology. *Toxicol. Sci.* **51,** 178–183

The authors wrote this commentary as a result of their participation as panelists in a poster-discussion session on the complementarity and usefulness of in vitro and in vivo approaches to neurotoxicity testing. The session was held in the 1998 meeting of the Society for Toxicology. This paper served as a catalyst for the present volume.

Trotti, D., Danbolt, N. C., and Volterra, A. (1998). Glutamate transporters are oxidant-vulnerable: a molecular link between oxidative and excitotoxic neurodegeneration? *Trends Pharmacol. Sci.* **19,** 328–334

The authors discuss the idea that glutamate transporters in the brain can be oxidized by biological agents, leading to decreased glutamate uptake and extracellular accumulation of neurotoxic glutamate. This phenomenon is of interest to neurotoxicologists in that a similar process can occur when cells are exposed to chemical oxidants. The possible involvement of oxidative alterations of specific glutamate transporters in pathologies (amyotrophic lateral sclerosis, Alzheimer's disease, brain trauma, and ischaemia) is reviewed.

METHODS AND MODEL SYSTEMS APPLICABLE TO IN VITRO NEUROTOXICOLOGY

Aschner, M., Kimelberg, H. K., and Vitarella, D. (1995) Selective techniques designed to evaluate neurotoxicity, in *Neurotoxicology: Approaches and Methodologies* (Chang, L. W., ed.), Academic, New York, pp. 439–444

This chapter presents a new version of an available technique for dynamic measurement of changes in cell volume of substratum-attached monolayer cell cultures. When combined with release measurements of endogenous cell markers, it affords a powerful tool for rapid measurements of cytotox-

icity and, potentially, a high throughput screening method for various neurotoxicants.

Banker, G. and Goslin, K. (eds.) (1998) *Culturing Nerve Cells*, 2nd ed., MIT Press, Cambridge, MA

This manual, now in its second edition, offers an outstanding resource for culture of vertebrate neural tissue and cells. It contains several eloquently written chapters on underlying principles, as well as detailed recipes and protocols for culturing specific cell types and mixed cultures. Contributors provide first-hand tutorials on the techniques developed in their laboratories, including advantages, limitations, and troubleshooting.

Boulton, A. A., Baker, G. B., and Bateson, A. N. (eds.) (1999) *In Vitro Neurochemical Techniques*, Humana, Totowa, NJ

This collection of contemporary techniques for neurochemical and molecular neurobiology research includes assays that are useful for the measurement of cell injury and cell death.

Buznikov, G. A., Nikitina, L. A., Bezuglov, V. V., Lauder, J. M., Padilla, S., and Slotkin, T. A. (2001) An invertebrate model of the developmental neurotoxicity of insecticides: effects of chlorpyrifos and dieldrin in sea urchin embryos and larvae. *Environ. Health Perspect.* **109,** 651–661.

This article describes an interesting new approach to in vitro developmental neurotoxicity testing that utilizes an invertebrate model.

Ehrich, M. (1998) Human cells as *in vitro* alternatives for toxicological research and testing: neurotoxicity studies. *Comments Toxicol.* **6,** 189–197

Sources of cells as well as advantages and disadvantages of the use of cells of human origin are discussed.

Freshney, R. I. (2000) *Culture of Animal Cells: A Manual of Basic Technique*, 4th ed., Wiley–Liss, New York

This book well deserves its common aphorism as the bible of tissue culture users. It is thorough, clearly written, well and generously illustrated, and frequently updated. Included are theoretical and practical considerations of cell, tissue, and organ culture, as well as detailed protocols of common procedures, such as sterile technique, preparation of medium, cytotoxicity assays, and cryopreservation. Detailed protocols are provided for two types of nervous system culture (cerebellar granule neurons and olfactory bulb ensheathing cells), but more specialized works would need to be consulted for other culture protocols.

Gad, S. C. (2000) Neurotoxicology *in vitro*, in *In Vitro Toxicology*, 2nd ed., (Gad, C. G., ed.), Taylor & Francis, New York, pp. 188–221

This article surveys the tools of in vitro neurotoxicology, including types of cell culture preparation used, specific examples of their use, methods of tissue culture, morphological and functional toxicity assays, and the design of neurotoxicant screening systems. Specific neurotoxicologic studies are briefly discussed as examples to illustrate the problems and potential advantages of in vitro approaches. the subjects selected are anticonvulsants, heavy metals, and excitoxins, mostly from articles published in the 1980s.

Gilbert, M. E. (2000) *In vitro* systems as simulations of in vivo conditions: the study of cognition and synaptic plasticity in neurotoxicology. *Ann. NY Acad. Sci.* **919,** 119–13.

The effects of regional brain stimulation and ablation on behavior have led to inferences on the impact of these manipulations on psychological constructs of "learning" and "memory." This review describes how an electrophysiological property, long-term potentiation (LTP), greatly expanded the ability to probe cellular aspects of the representation of memories in the brain. The study of plasticity in this manner is an excellent example of how in vivo phenomena translate to more simplified in vitro test systems to directly address cellular and biochemical mechanisms of information storage in the brain.

Maines, M., Costa, L. G., and Reed, D. J. (eds.) (2002) *Current Protocols in Toxicology* Wiley, New York

This two-volume collection offers detailed laboratory procedures for the assessment of toxicity at multiple levels of biological complexity ranging from whole organisms to biochemical pathways. It is available in updatable looseleaf, CD-ROM, and Web-based formats. Chapter 12, "Biochemical and Molecular Neurotoxicology," contains units written by authorities in their fields on several in vitro topics, including the development of an in vitro blood–brain barrier, culture of rat hippocampal neurons and rat cortical astrocytes, and analytical techniques for cytology and imaging.

O'Hare, S. and Atterwill, C. K. (eds.) (1995) *In Vitro Toxicity Testing Protocols*, Humana, Totowa, NJ

This collection of detailed protocols includes the preparation and use of cultured astrocytes for assays of gliotoxicity, as well as several chapters on general and topical toxicity.

Tyson, C., Witschi, H., and Frazier, J., (1994) *In Vitro Toxicity Indicators*, Methods in Toxicology Vol. 1B, Academic, New York

This book contains detailed testing procedures for assessing cell injury and cell death. The chapters do not specifically address neural cells, but protocols can be adapted for use in neurotoxicity testing.

Zurich, M. G., Honegger, P., Schilter, B., Costa, L. G., and Monnet-Tschudi, F. (2000) Use of aggregating brain cell cultures to study developmental effects of organophosphorus insecticides. *Neurotoxicology* **21,** 599–606

This experimental study shows how aggregating cultures of brain cells can be used to assess developmental neurotoxicity.

REVIEWS ON NEUROTOXIC SUBSTANCES STUDIED IN VITRO

Costa, L. G., Guizzetti, M., Lu, H., Bordi, F., Vitalone, A., Tita, B., Palmery, M., Valeri, P., and Silvestrini, B. (2001) Intracellular signal transduction pathways as targets for neurotoxicants. *Toxicology* **160,** 19–26

This review focuses on the interactions of lead, ethanol, and polychlorinated biphenyls with signal transduction pathways, especially protein kinase C isoenzymes. The potential importance of such pathways in neurotoxic processes is discussed.

Gilbert, M. E. and Lasley, S. M. (2002) Long-term consequences of developmental exposure to lead or polychlorinated biphenyls: synaptic transmission and plasticity in the rodent CNS. *Environ. Toxicol. Pharmacol.* **12,** 105–117

The authors review current evidence concerning the effects of exposure to lead or polychlorinated biphenyls (PCBs) on hippocampal synaptic transmission and use-dependent plasticity, particularly effects that persist long after exposure has ended. Long-term potentiation (LTP) is thought to represent a physiological substrate for memory, and during ontogeny, this type of plasticity guides the establishment and maintenance of synaptic connections in cortical structures. It is proposed that in the developing nervous system PCB or lead perturb activity-dependent plasticity leading to organizational changes in brain. The aberrant connectivity resulting during development is manifested as impaired LTP and cognitive ability in the mature organism.

Guerri, C., Pascual, M., and Renau-Piqueras, J. (2001) Glia and fetal alcohol syndrome. *Neurotoxicology* **22,** 593–559

The article reviews evidence obtained in vivo and in culture that ethanol directly damages astrocytes and radial glia, impairing neuronal migration in the developing brain.

Kodavanti, P. R. S., and Tilson, H. A. (2000). Neurochemical effects of environmental chemicals: *in vitro* and *in vivo* correlations on second messenger pathways. *Ann. NY Acad. Sci.* **919,** 97–105

This article focuses on correlating changes in second-messenger pathways following in vitro and in vivo exposure to persistent environmental chemicals such as polychlorinated biphenyls (PCBs). Second messengers, including calcium, protein kinase C, and inositol phosphates, are critical for nervous system development and function. This article reports changes in these pathways in in vitro neuronal cultures at concentrations that are biologically relevant.

Tiffany-Castiglioni, E., Legare, M. E., Schneider, L.A., Hanneman, W.H., Zenger, E., and Hong, S. (1996) Astroglia and lead neurotoxicity, in *The Role of Glia in Neurotoxicity* (Aschner, M. and Kimelberg, H. K., eds.), CRC, Boca Raton, FL, pp. 175–200

This chapter reviews in vivo and in vitro work on the effects of lead on mammalian astroglia dating from 1993 to 1996. Topics include calcium homeostasis, glutathione metabolism, morphology, and cytoskeletal proteins.

Tiffany-Castiglioni, E. (1993) Cell culture models for lead toxicity in neuronal and glial cells. *Neurotoxicology* **14,** 513–536

This article critically reviews most of the work to 1993 on the effects of lead on astroglia, oligodendroglia, Schwann cells, and neurons in culture.

Mammalian and invertebrate models are included. The work reviewed is organized historically into three phases: the exploratory, expansion, and intensification stages of in vitro lead neurotoxicology. These phases are characterized by progressive refinement of end points from lethal responses at millimolar doses to physiologically relevant molecular responses at submicromolar doses. The article also contains a still timely detailed discussion on problems with lead concentrations and exposure protocols in in vitro.

Veronesi, B., Ehrich, M., Blusztain, J. K., Oortgiesen, M., and Durham, H. (1996) Cell culture models of interspecies selectivity to organophosphorus insecticides. *Neurotoxicology* **18,** 283–298

The article presents an integrated summary of studies by the authors in which interspecies differences in responses of nervous tissue to organophosphorus insecticides were examined in vitro. By the use of human and mouse cell lines, as well as homogenized tissue, the underlying mechanisms for interspecies differences were shown to include targets not previously recognized in vivo, including cellular metabolism, target enzyme baseline activities, and receptor-medicated cell-signaling pathways.

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