Delineating Toxicity Pathways of Peripheral Neuropathy

David Herr, Ph.D.
Neurotoxicology Division
US EPA
RTP, NC
McKim Conference on Predictive Toxicology

What is Neurotoxicity?

- **Neurotoxicity**: “An adverse change in the structure or function of the central and/or peripheral nervous system following exposure to a chemical, physical, or biological agent”

- **Adverse Effect**: “Alterations from baseline or normal conditions that diminish an organism’s ability to survive, reproduce, or adapt to the environment”

1Guidelines for Neurotoxicity Risk Assessment
Federal Register, 63(93):26926-26954, 1998
http://cfpub.epa.gov/ncea/raf/recorddisplay.cfm?deid=12479
Complexity of Nervous System

- Peripheral Nervous System (PNS)
  - Peripheral Nerves
  - Neuromuscular Junction
- Autonomic Nervous System (ANS)
- Central Nervous System (CNS)

- Characteristics that increase vulnerability
  - High energy requirements
  - Electrical transmission of action potentials and chemical transmission
  - Long spatial extensions (axons) and large cell volumes
    - Need to transport cellular material

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Signs of Peripheral/Central Neuropathies

- Biochemical
  - Neurofilament accumulations
  - Changes in axonal transport
  - Altered myelin
  - Altered ion gradients

- Physiological
  - Decreased amplitudes of nerve action potentials
  - Decreased nerve conduction velocity
  - Denervation potentials in muscles
  - Alterations in somatosensory evoked potentials

- Behavioral
  - Parasthesias
  - Increased reaction time
  - Vibrotactile abnormalities
  - Paralysis

- Pathological
  - Neuronal / Axonal degeneration
  - Changes in myelin
Systems Biology: Define normal function and its bounds, where further insult compromises function and leads to toxicity

**Patterns of Neurotoxic Injury**

- **Neuronopathy**: Death of entire neuron  
  - Astrocyte proliferation

- **Axonopathy**: Axon degenerates  
  - Neuronal chromatolysis  
  - Nissl substance and nucleus to periphery

- **Myelinopathy**: Injury to Schwann or Oligodendrocytes
Types of Toxicity – Neuronopathies

• Primary “Neuronal” Site of Action
  – Metabolic Inhibitors: 6-Amino-nicotinamide, arsenic
  – Inhibit DNA/Protein Synthesis: cloramphenicol, doxorubicin
  – Protein Binding: thallium, methyl mercury (?
  – Excitotoxicity: B-N-Methylamino-L-alanine (BMAA)

• Multiple chemical classes, Modes of action(s)
  – No single event leads to neuronal death
  – Overwhelm repair process = f.(dose, duration, persistence, repair)
  – Can model NMDA receptors, metabolic inhibitors (?),
    propensity for protein binding (?), etc...
  – What is critical event to produce neuropathy?

Types of Toxicity - Axonopathies

• Classical Agents:
  • Gamma-Diketones
    (Hexane, 2,5-Hexanediione, n-butyl ketone)
  • Carbon Disulfide
  • B,B′-Iminodipropionitrile
  • Acrylamide
  • Zinc Pyridinethione

Adapted From: Anthony et al., Cassarett & Doull, 2001
Mechanisms of PNS Toxicity - 
*n*-hexane and CS₂

- Gamma-Diketone reacts with amino groups
- Pyrrole Formation
- Pyrrole Oxidation
- Covalent Protein X-Linking

CS₂ reacts with amino groups
Dithiocarbamate adducts with lysyl amino groups
Isothiocyanate adducts react with protein nucleophiles
**N,S-dialkyldithiocarbamate esters are slowly reversible**
Thiourea adducts are irreversible

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Mechanisms of PNS Toxicity - 
OPIDN

- Irreversible = OPIDN
- Reversible = No OPIDN

**Group A:**
Phosphonates = phosphorofluoridates = phosphonofluoridates = phosphorodiaminodifluoridates = phosphoroamidofluoridates > phosphates > phosphorotrithioates > phosphorothioates = phosphonothioates = phosphinothioates > phosphorochloridates

Adapted From: O’Callaghan, 2003
Winthraw et al., 2003
Massicotte, 1998
Chambers and Levi, 1992
Mechanisms of PNS Toxicity - Axonopathy

- Primary “Axonal” Site of Action
  - Neurofilament Cross-Linking: 2,5-Hexanedione, 3,4-dimethyl-hexanenedione, CH₃ n-butyl ketone, CS₂
    - Distal -> central
  - Altered Axonal Transport: IDPN (anteriorgrade), pyridinethione (retrograde), acrylamide? (nerve terminal)
  - Organophosphate-Induced Delayed Neuropathy: Tri-ortho-cresyl phosphate (TOCP), O-ethyl O-4-nitrophenylphenylphosphonothioate (EPN), leptophos
    - Neuropathy Target Esterase (NTE) – Requires aging?, delayed for 7-10 days (after cholinergic signs)
  - Actions on Microtubules: Colchicine, vincristine, vinblastine, paclitaxel
    - Bind to tubulin, depolymerization of microtubules (or stabilization – paclitaxel)

- Perhaps the best predictive capabilities

Mechanisms of PNS Toxicity - Myelinopathy

- Primary “Glial” Site of Action
  - Intramyelinic Edema: Hexachlorophene, acetyllethyltetramethyl tetralin (AETT), ethidium bromide, triethyltin
    - Splitting of intraperiod line (PNS and CNS), Spongiosis of brain
  - Demyelination: Tellurium, amiodarone, disulfiram, lysolecithin, perhexilene, lead
    - Schwann cells lose ability to maintain myelin and/or die
    - Disassemble concentric layers of myelin
    - Largest axons > smaller axons
  - Metabolic Disruptors: 6-ANT, Metronidazole, fluoroacetate/fluorocitrate, methionine sulfoximine, disulfiram
    - Disrupt mitochondrial metabolism, TCA cycle, ATP production, glutamine synthetase, chelates cations
    - Altered glutamine metabolism
**Challenges for the Future – Systems Biology and QSAR**

- System-level understanding of biological processes
  - Initiating events and subsequent downstream critical events
- Model Components:
  - Structure of the system (gene regulatory systems, biochemical networks, and physical structures)
  - Dynamics (changes over time) of the system, both quantitatively and qualitatively
    - Theory/model needs predictive capability
    - Biological controls of the system
- Appropriate tools to design the system
Moving to the Future - Modeling Strategies

Physiological Systems & Function

Top-Down

Systems → Organs

Middle-Out

Tissues ← Pathways

Bottom-Up

Organelles → Cells

Molecular Data & Mechanisms

Modified From: Noble, 2003

Thanks for your time and attention!
**Toxic Pathway(s)**

- Metabolic inhibitors
- DNA/Protein synthesis/function
- Oxidative stress
- Excitotoxicity
- Neurofilament X-linking
- Axonal transport
- Lack of “cell constituents”
- Ion chelation
- Loss of ion gradients – mitochondrial uncoupling
- Altered lipid metabolism
- Metabolic disruption
- Altered glutamine metabolism
- Membrane turnover
- Inhibition of NTE
- Membrane turnover
- Inhibition of NTE
- Ion chelation

**Types of Validity**

- **Content Validity**: Does the effect result from exposure to a substance (dose-related)?

- **Construct Validity**: Does the test measure what we think it does? Is the effect adverse or toxicologically relevant?

- **Concurrent Validity**: Correlative measures among other endpoints (biochemical, physiological, behavioral, pathological)

- **Predictive Validity**: Do the results predict what will happen under various conditions? Are the results predictive of human effects?