Achieving The Goals Of Toxicity Testing In the 21st Century: The TestSmart Developmental Neurotoxicology (DNT) Testing Program

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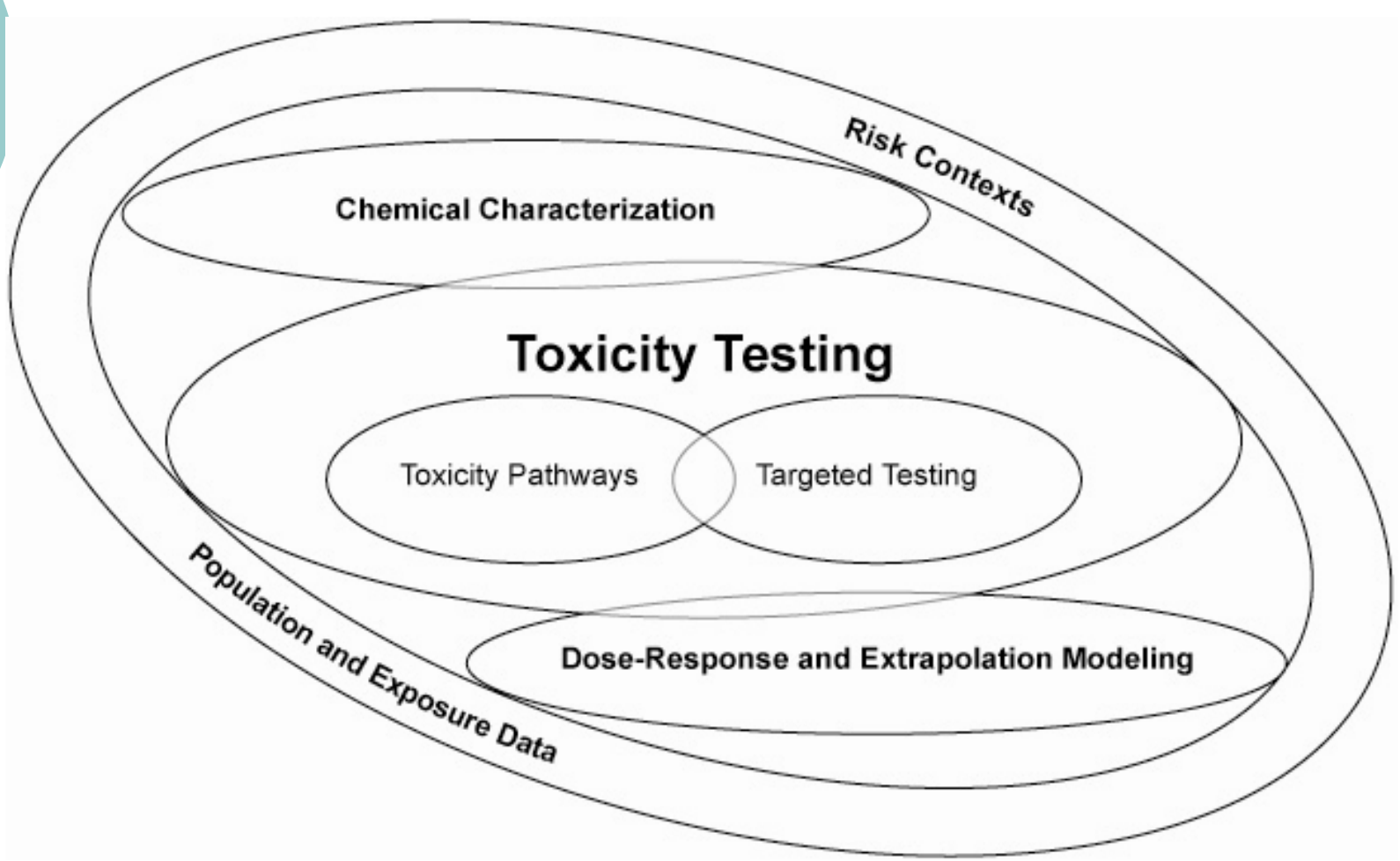
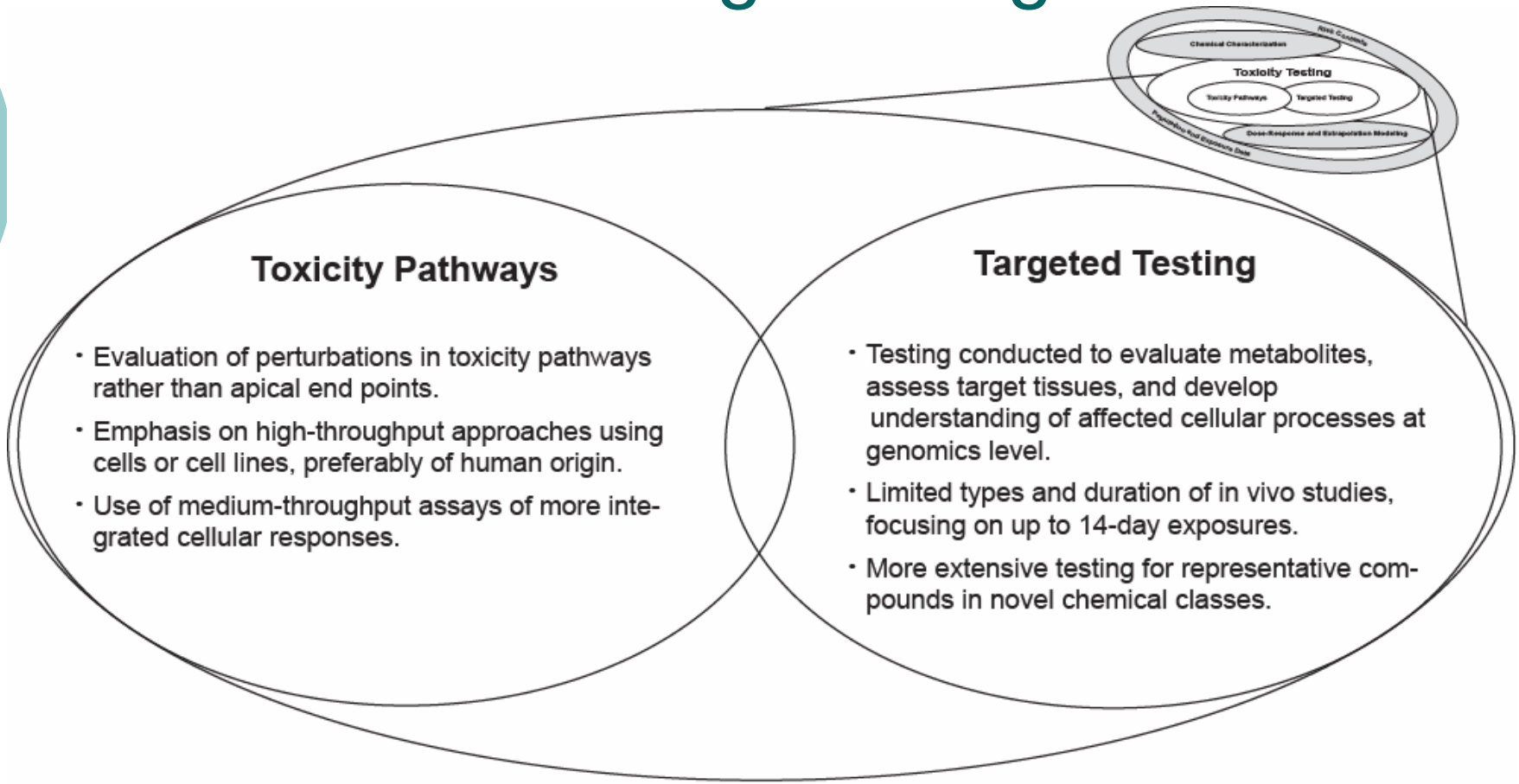
A Framework for Toxicity Testing in the 21st Century

Components Of The Vision



Chemical Testing Strategies



Toxicity Pathways

There are unique pathways in the central nervous system

Toxicity Pathways In Report (Pathways sensitive to toxicants)

- NrF2-most types of cells
- Nuclear receptor-peroxisome, pxr, car
- DNA repair
- Sex steroids
- Hypo-osmolarity

Relatively Specific Neurotoxicity Pathways (intracellular)

- Ion channels-potassium, sodium, chloride, glutamate, gamma-aminobutyric acid
- Lipid biosynthesis (myelin)
- Axonal transport
- Synaptic vesicle recycling

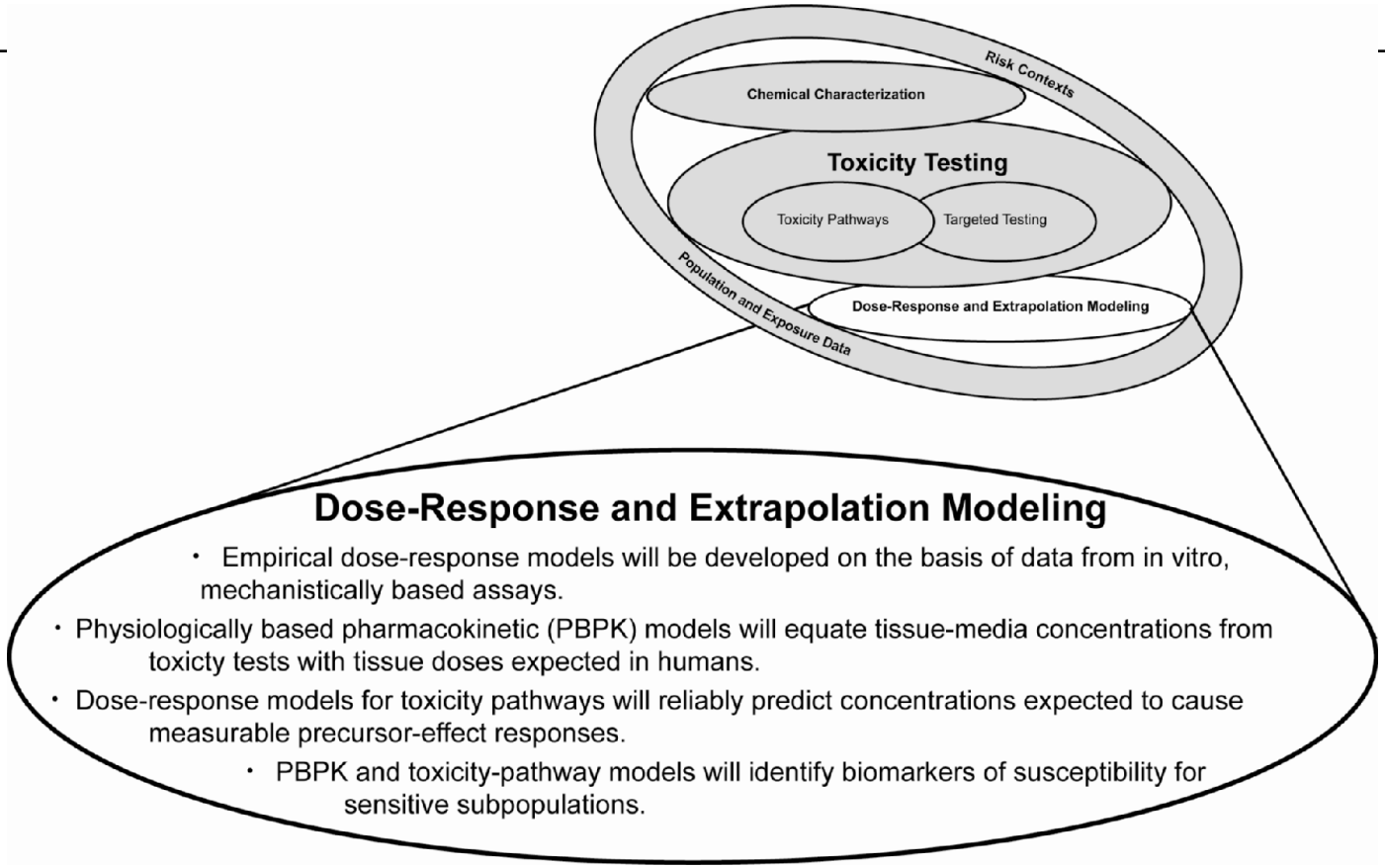
Toxicity Pathways (Cell-to-Cell)

- Oligodendrocyte myelinate axons
- Neuron:neuron interaction in forming synapses
- Astrocyte:neuron interactions forming and eliminating synapses
- Astrocyte:endothelial interaction forming blood brain barrier

Regional and Inter-regional Pathways

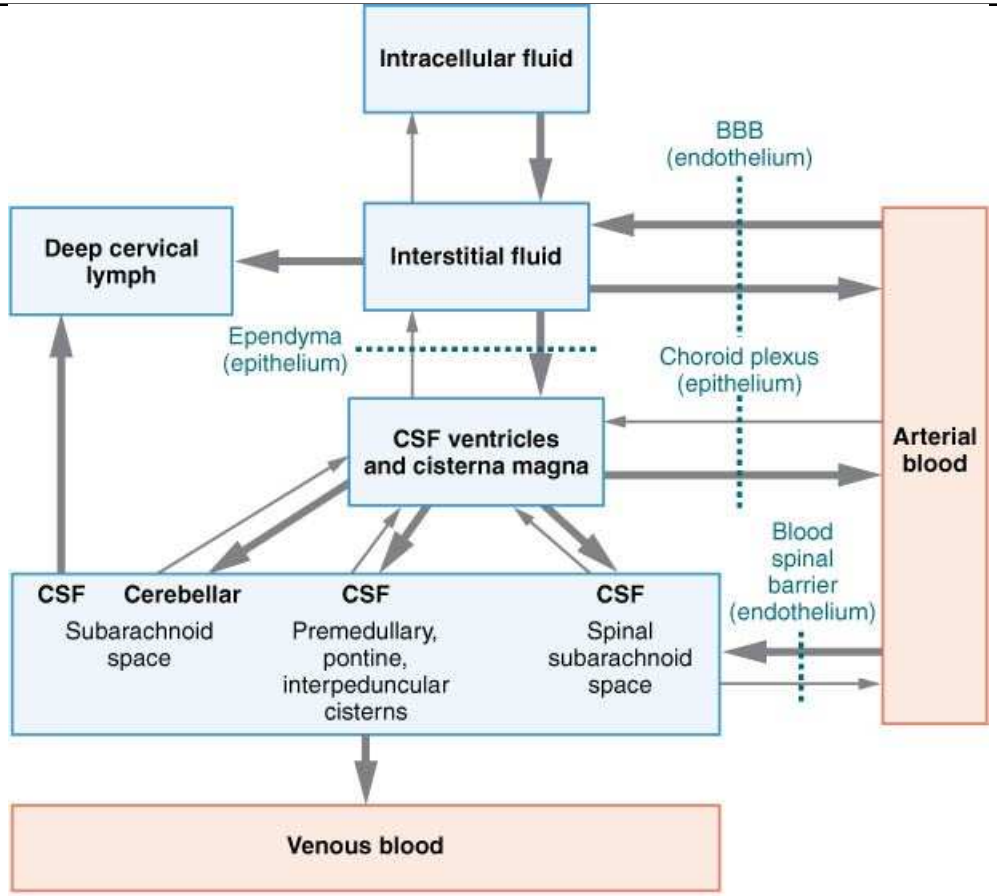
- Hippocampal pathways
- Ascending and Descending spinal pathways
- Thalamocortical pathways
- Corpus collasum

Predicting Dose



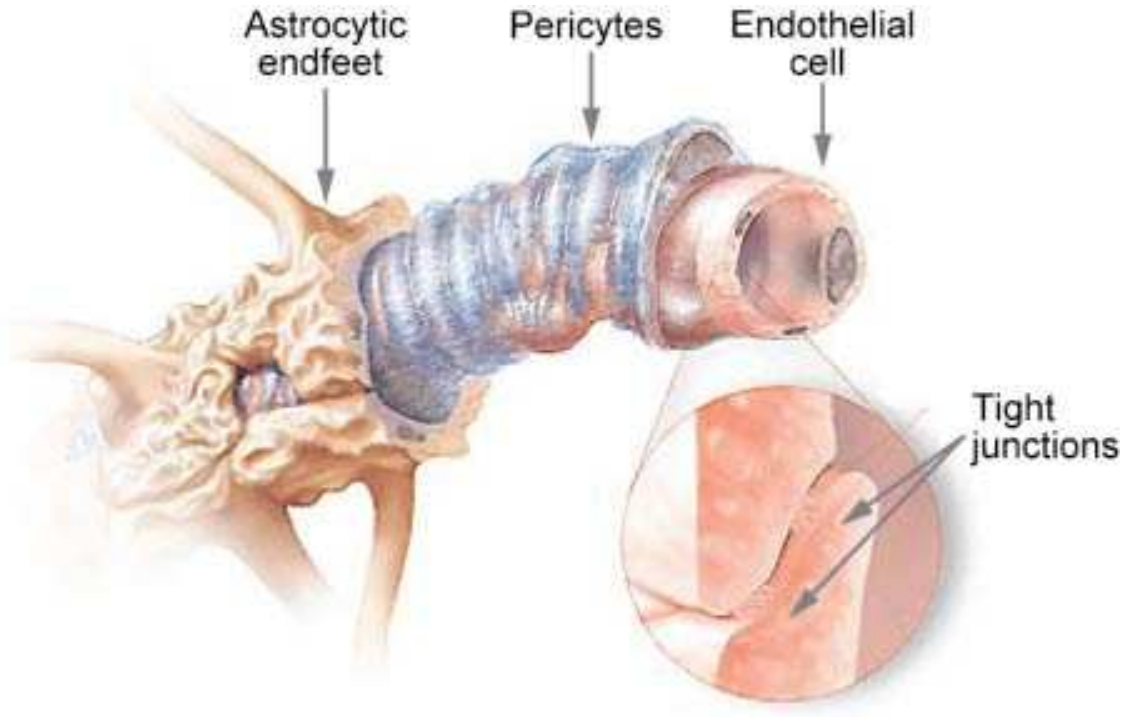
"All substances are poisons; there is none which is not a poison. The right dose differentiates a poison…." Paracelsus (1493-1541)

Toxicokinetics Unique In The CNS



de Boer AG, Gaillard PJ. 2007.
Annu. Rev. Pharmacol. Toxicol. 47:323–55

Blood Brain Barrier Is Tight (Very High Electrical Resistance)



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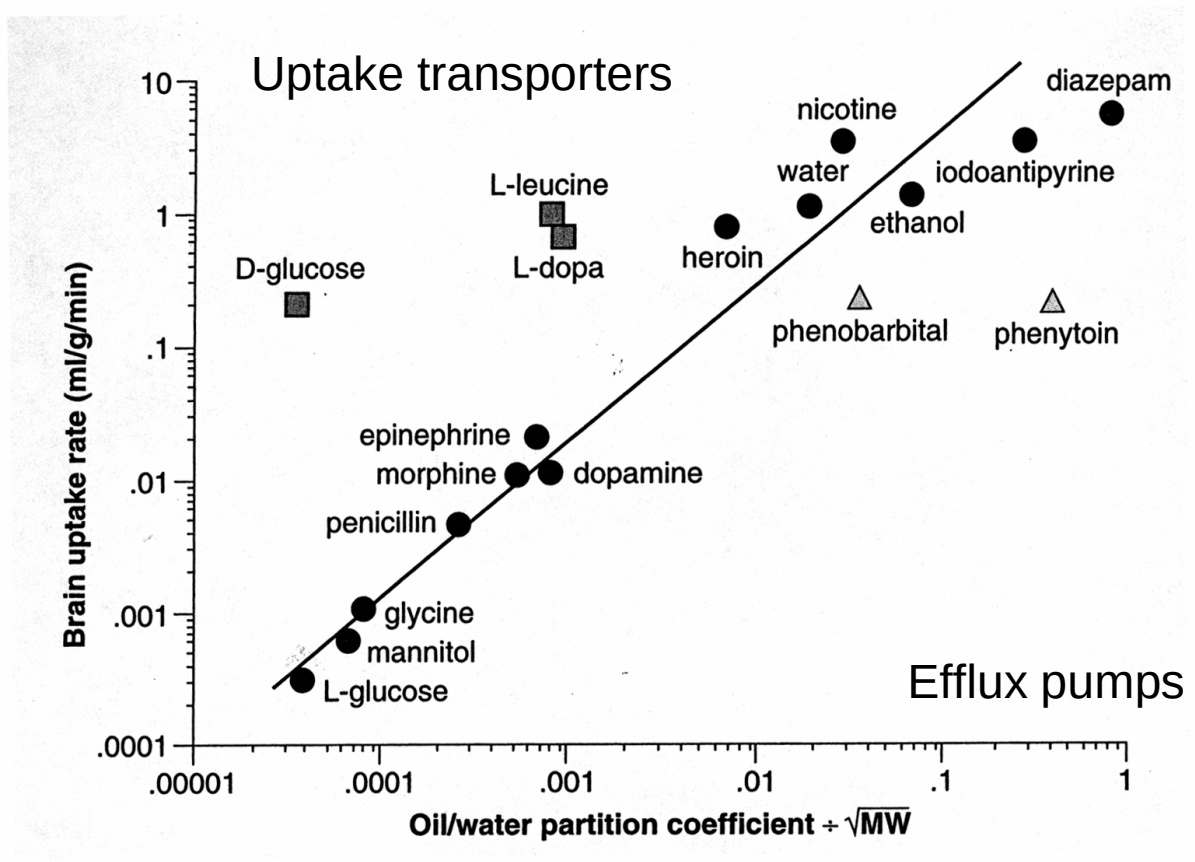
Electrical Resistance of Cell Lines

CELL MODEL	Ohms/cm
MDCK	1000-1200
Caco-2	400-600
BrainEndo/astro/peri	50-600

Do We Need An In Vitro Model For the BBB?

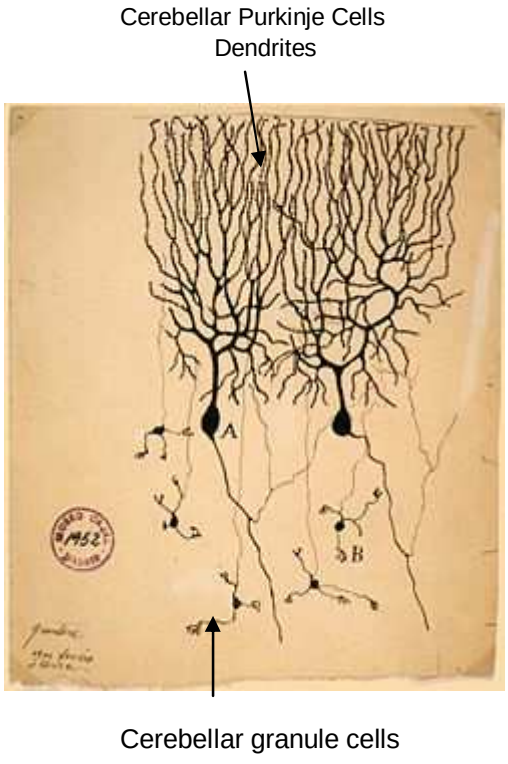


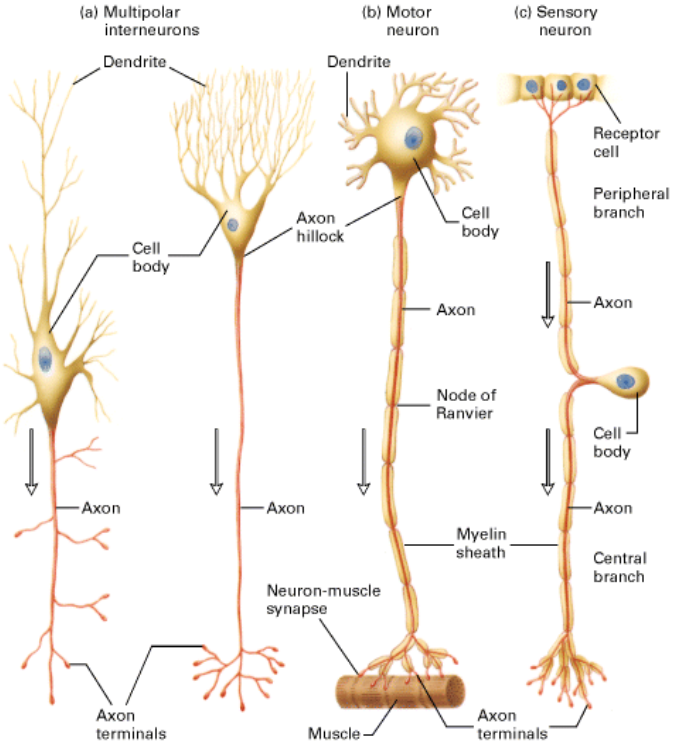
QSAR Model For Blood Brain Permeability?

Report Emphasizes Human Cell Lines

- Neuroblastoma cell lines derived from neural crest
- Adult neural stem cells (limitations)
- Glial cell lines most from brain tumors, many GFAP expressing none (to my knowledge) express oligodendroglial properties
- NT-2 teratocarinoma cell line
- Human embryonic stem cells (limitations)

Is It Possible To have A Prototype Neuronal Cell Line?





Computer Models-Systems Biology

- Mammalian thalamocortical system
- Deep brain stimulation
- Blue Brain Project

TestSmart DNT I Developed Recognizing Problems

- Objective to bring together stakeholders (test developers, user, regulators and advocates, toxicologists/neuroscientists) to discuss the scientific issue and policies concerning developmental neurotoxicology
- Held in Reston, VA, 2006
- Organized by CAAT, NTP, and US EPA,

TestSmart DNTI Recommendations

- **There is a need for more efficient models;**
  - **systematic analysis of current models;**
  - **short-term identify models;**
  - **high-through put models;**
- **DATA;**
  - **structure for collecting data**
  - **reference chemicals;**
  - **a decision framework;**
  - **continue dialogue**
  - **resources for support**

- Lein et al., EHP 115:764

TestSmart DNT II (Objectives)
November 12-14, 2008, Reston, VA

- Policy framework (applying DNT data)

  establishing priorities for further action on the basis of DNT data

  using data from alternative tests in conjunction with data from, e.g., animals and humans

  using data from alternative tests when other data is not available

DNT II (Objectives)

- Biology Framework

  establishing and codifying criteria for evaluating alternative methods

  determining the usefulness of current methods

  highlight areas that need more research (methods, models, endpoints, new biology)

How Will The Objectives Be Accomplished

- Discuss the endpoints

  1) cell proliferation, differentiation, cell death

  2) neuronal connectivity, neurite outgrowth, synapse formation and function

  3) glial function-guidance, migration, myelination, vascular, inflammation

How Will The Objectives Be Accomplished

- Discuss the Models

  cell culture-neuronal and glial cell lines, primary cultures, and stem cells

  non-mammalian animals, zebra fish and nematodes

  Discuss the data-interpretation, integrating from different tests, and developing policy

  computation

  using in vitro data in understanding health effects and risk

All Are Welcomed

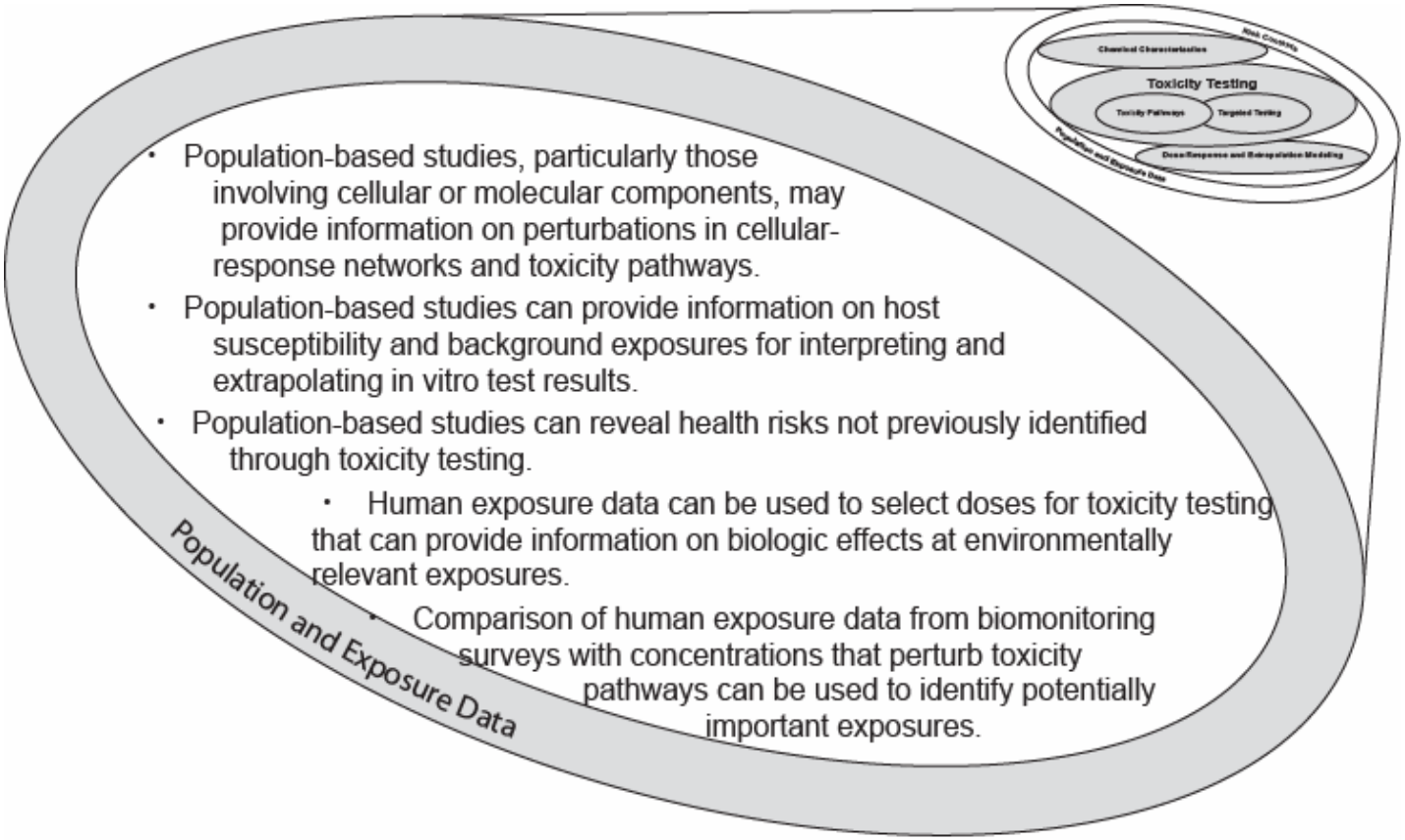
Acknowledgements

- Organizers of the meeting for the invitation to speak
- DNT organizers including Alan Goldberg, Pamela Lein, William Mundy, and Kevin Crofton

Organization

- **Day 1-plenary session discussing "Toxicity Testing in the 21 Century, adverse neurodevelopmental outcomes**
- **Day 1-endpoints**
- **Day 1-recap**
- **Day 2-plenary session on models**
- **Day 2-breakout groups on cell culture and models and summary**
- **Day 2-Talks on data (computation and interpretation)**
- **Day 2-Panel discussion on decision making**
- **Day 3-Evaluating the meeting-policy and biology framework. Summary and comments from all**

Report Emphasizes Population Studies



Assessing Neural Function In Humans (adults)

- **Neurodegeneration**

  assessment relatively easy when evaluating neurodegenerative disease

  relatively difficult if evaluating subtleties

  epidemiological studies on exposures are difficult due to length of time between exposure and phenotype

Assessing Neural Function In Humans (children)

- Assessment easy if evaluating severe outliers from normal development and relatively success in evaluating subtleties (Pb)

  exposure assessment can be difficult during gametogenesis during pregnancy.