

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

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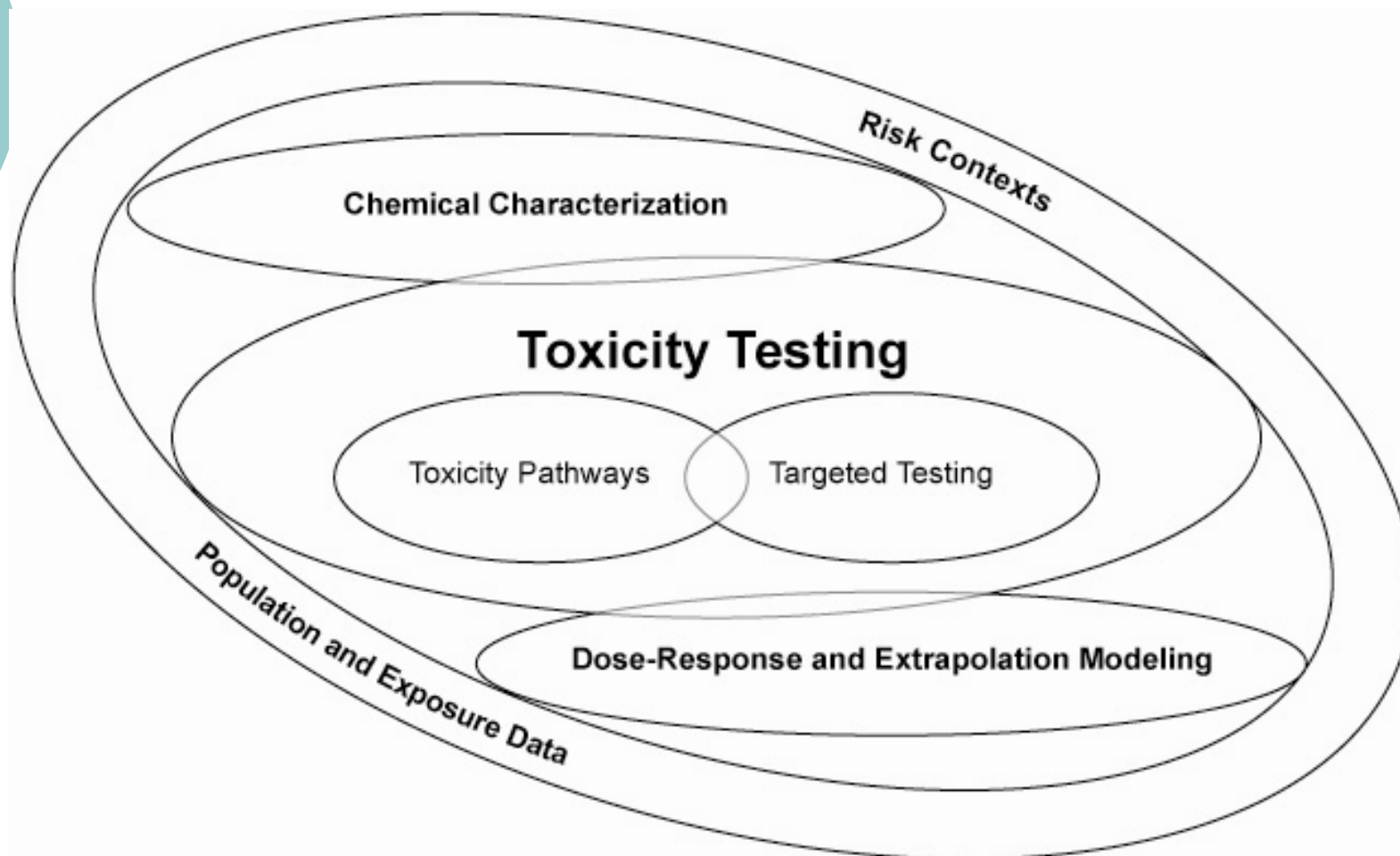


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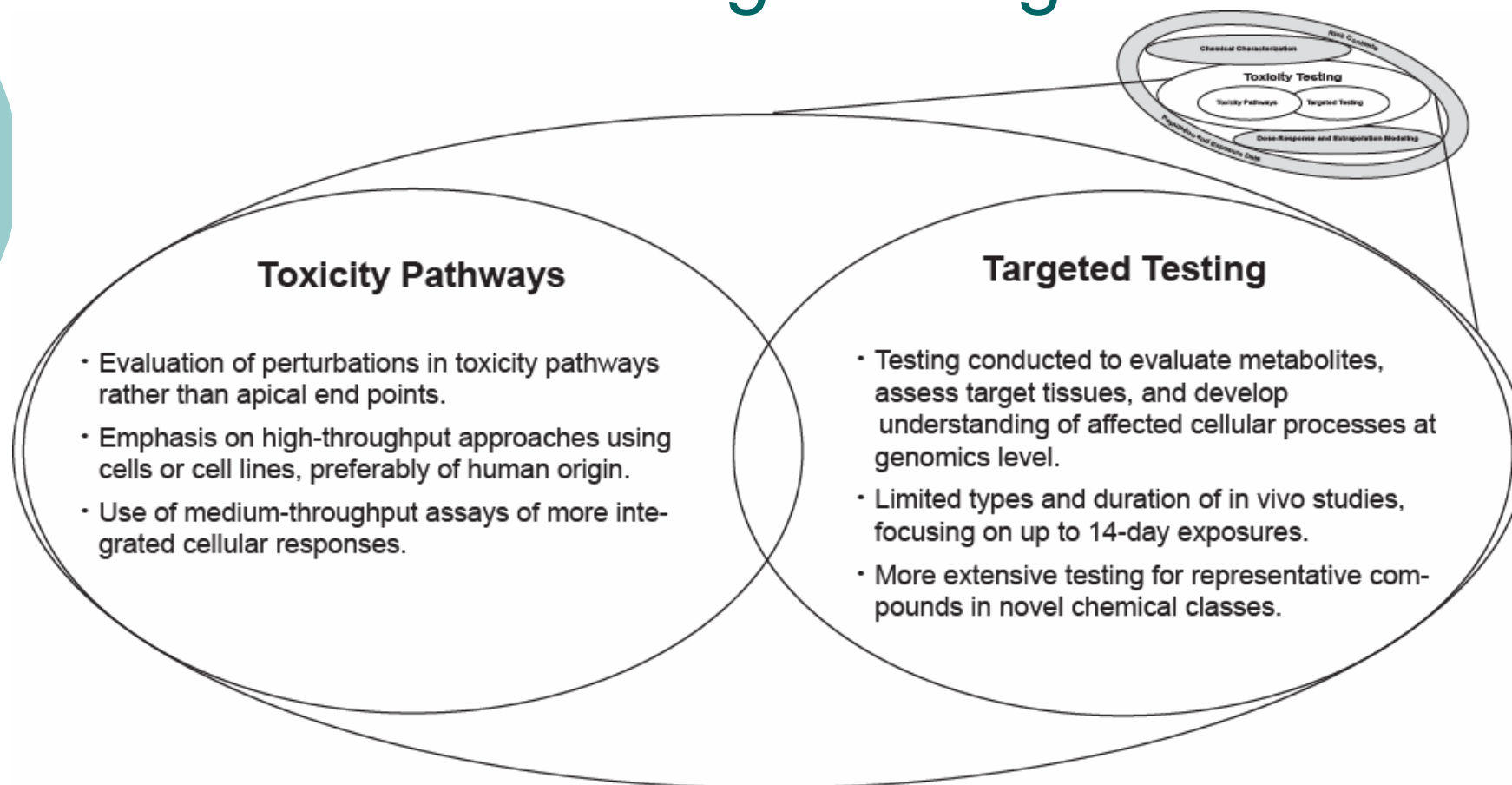
**A Framework  
for  
Toxicity Testing in the 21<sup>st</sup> Century**

# Components Of The Vision

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# Chemical Testing Strategies





# Toxicity Pathways

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There are unique pathways in the central nervous system



## Toxicity Pathways In Report (Pathways sensitive to toxicants)

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



## Relatively Specific Neurotoxicity Pathways (intracellular)

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



## Toxicity Pathways (Cell-to-Cell)

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



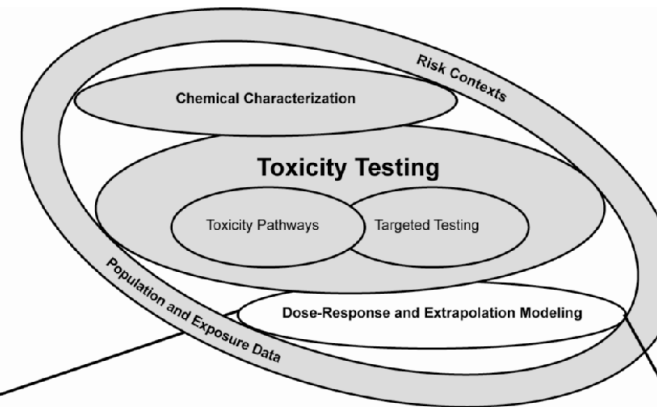


## Regional and Inter-regional Pathways

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

# Predicting Dose

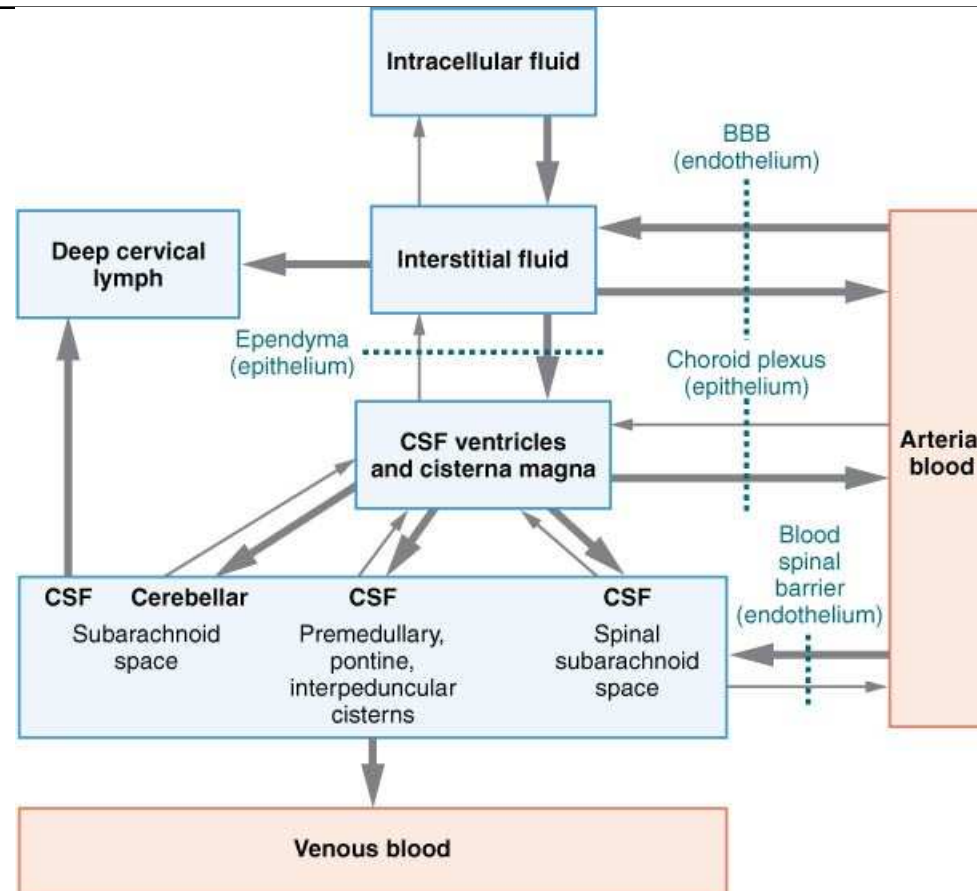


## Dose-Response and Extrapolation Modeling

- Empirical dose-response models will be developed on the basis of data from in vitro, mechanistically based assays.
- Physiologically based pharmacokinetic (PBPK) models will equate tissue-media concentrations from toxicity tests with tissue doses expected in humans.
- Dose-response models for toxicity pathways will reliably predict concentrations expected to cause measurable precursor-effect responses.
  - PBPK and toxicity-pathway models will identify biomarkers of susceptibility for sensitive subpopulations.

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

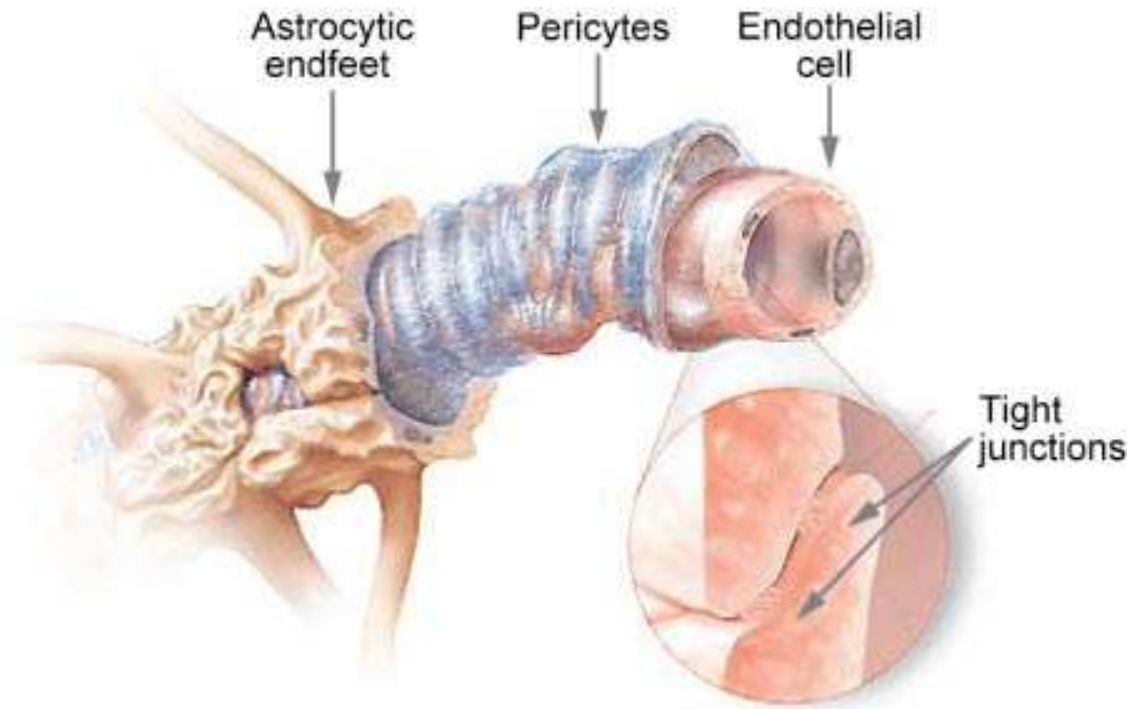
# Toxicokinetics Unique In The CNS



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

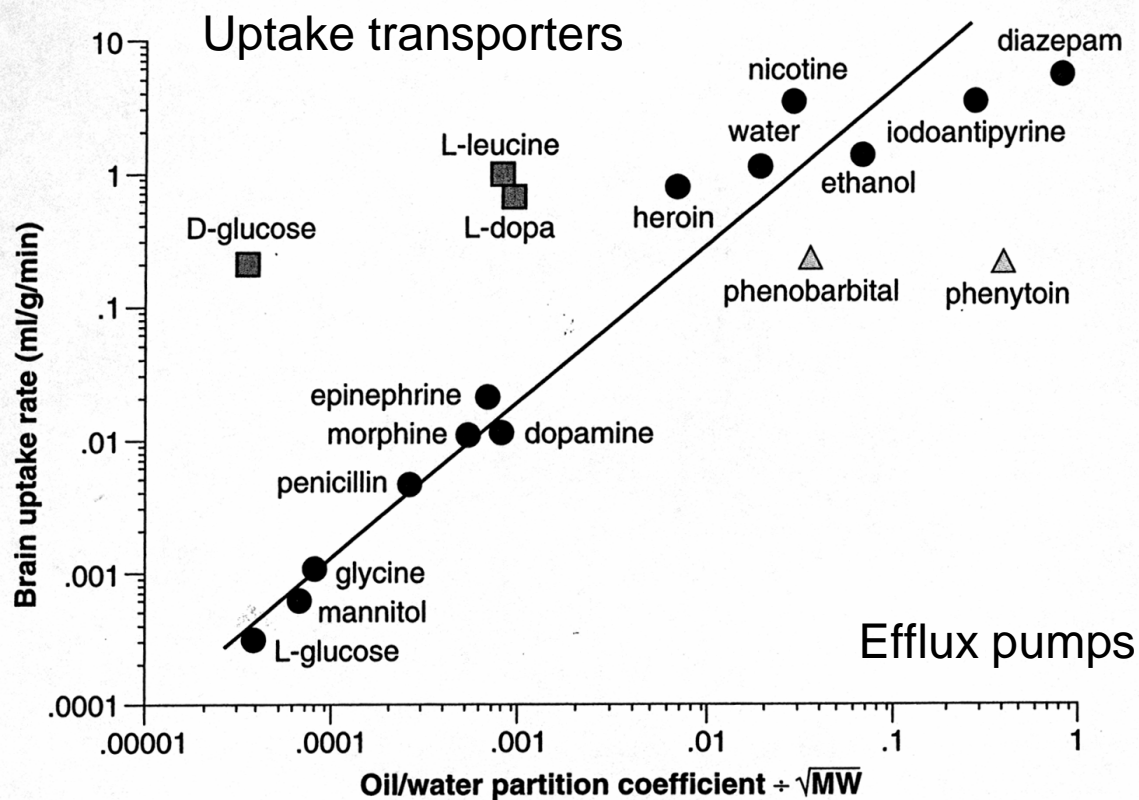


# Electrical Resistance of Cell Lines

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<u>CELL MODEL</u>	<u>Ohms/cm</u>
○ MDCK	○ 1000-1200
○ Caco-2	○ 400-600
○ BrainEndo/astro/p eri	○ 50-600

# Do We Need An In Vitro Model For the BBB?



QSAR Model For Blood Brain Permeability?

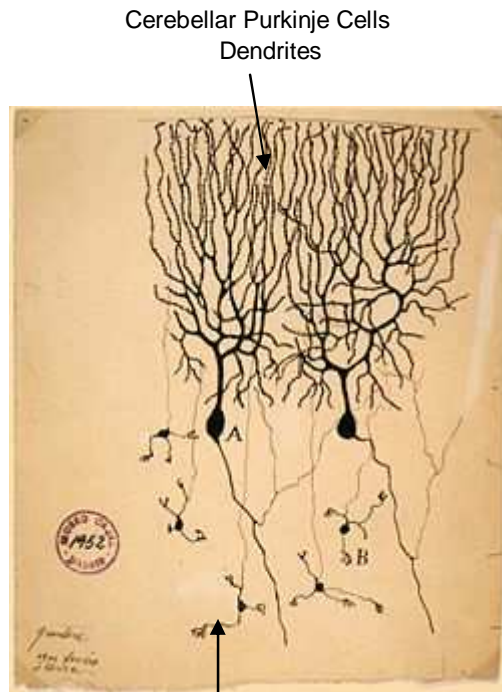


## Report Emphasizes Human Cell Lines

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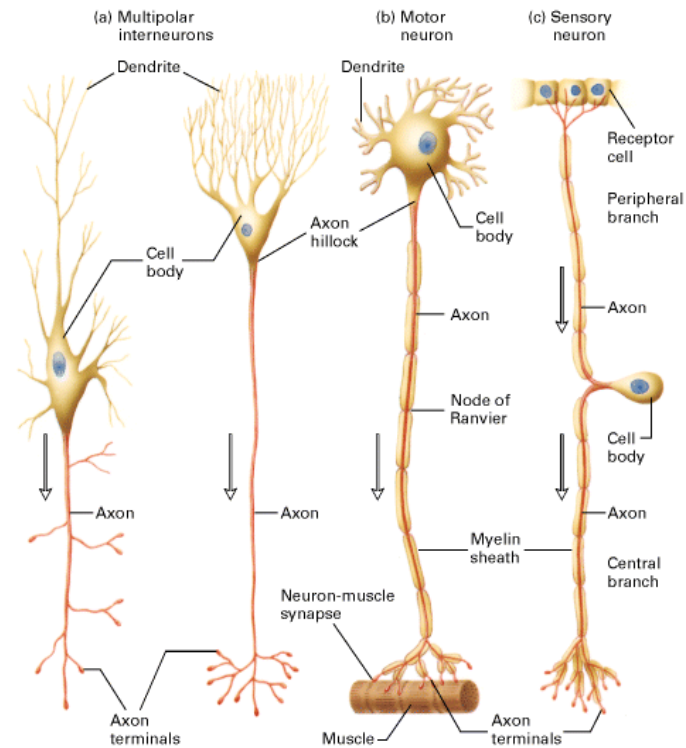
- Neuroblastoma cell lines derived from neural crest
- Adult neural stem cells (limitations)
- Glial cell lines most from brain tumors, many GFAP expressing none (to my knowledge) express oligodendroglial properties
- NT-2 teratocarcinoma cell line
- Human embryonic stem cells (limitations)

# Is It Possible To have A Prototype Neuronal Cell Line?



Cerebellar Purkinje Cells  
Dendrites

Cerebellar granule cells







# Computer Models-Systems Biology

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



# TestSmart DNT I Developed Recognizing Problems

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- 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

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- **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**



# TestSmart DNT II (Objectives)

November 12-14, 2008, Reston, VA

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- 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)

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- 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

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- 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

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- 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

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# Acknowledgements

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



# Organization

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- **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

- Population-based studies, particularly those involving cellular or molecular components, may provide information on perturbations in cellular-response networks and toxicity pathways.
- Population-based studies can provide information on host susceptibility and background exposures for interpreting and extrapolating in vitro test results.
- Population-based studies can reveal health risks not previously identified through toxicity testing.
  - Human exposure data can be used to select doses for toxicity testing that can provide information on biologic effects at environmentally relevant exposures.
  - Comparison of human exposure data from biomonitoring surveys with concentrations that perturb toxicity pathways can be used to identify potentially important exposures.

Population and Exposure Data





# Assessing Neural Function In Humans (adults)

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- **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)

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- 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.