In vitro and other methods for identifying developmental neurotoxicants

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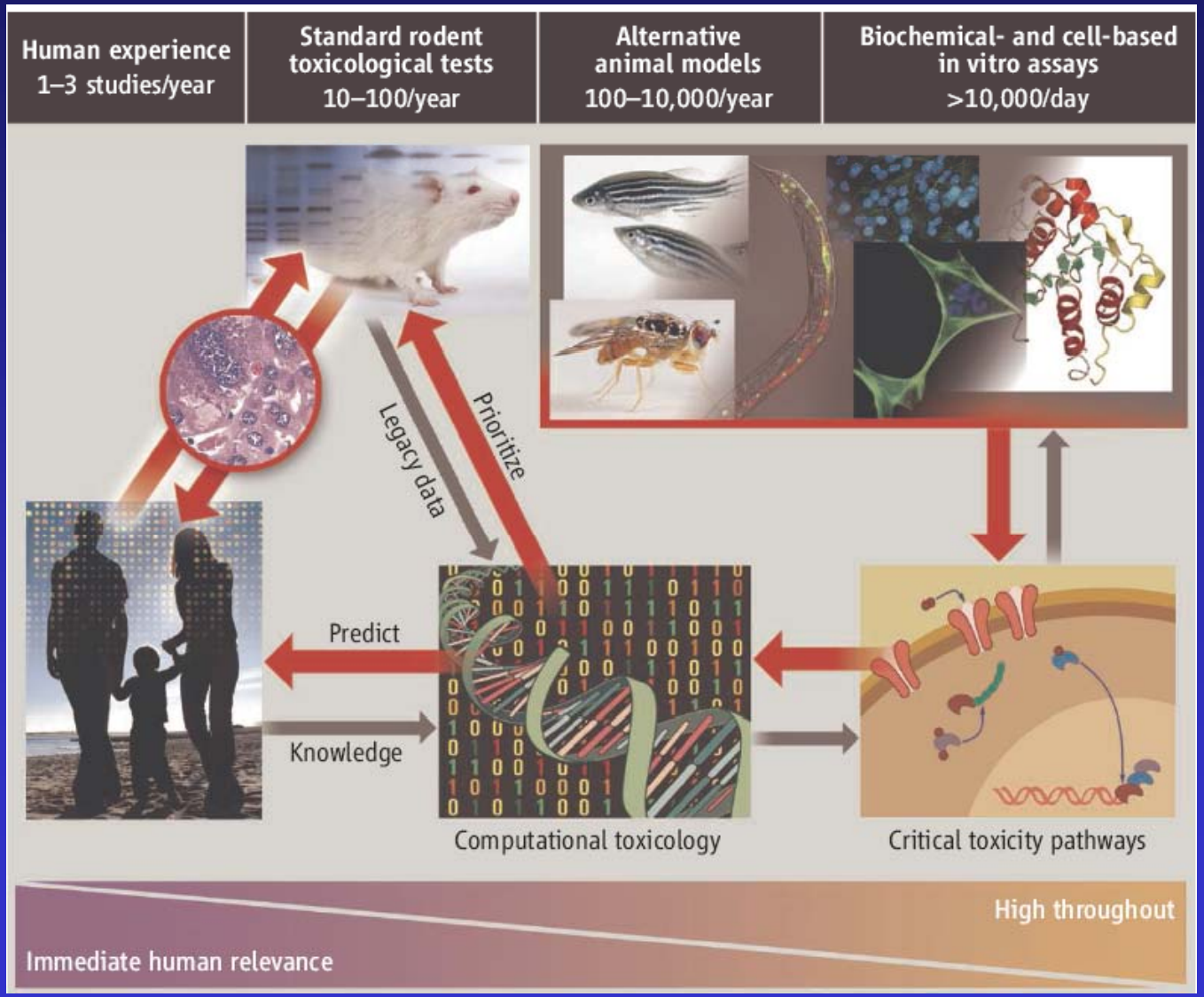
Current developmental neurotoxicity testing (DNT) approach *versus* reality

- **68,000 to 120,000 chemicals require toxicity testing under REACH legislation**
- **Using current testing protocols, estimated costs are**
  - 1.5 to 3 years to complete studies of one chemical
  - 54 million vertebrate animals
  - 9.5 billion euro
- **Current animal models are not consistently reliable predictors of human response**
  - Only 43% of toxic effects in humans correctly predicted by tests in rodents, 63% with inclusion of non-rodent animal tests
  - Typically test high doses that do not reflect human exposure
  - Test across multiple endpoints (increasing false positives)

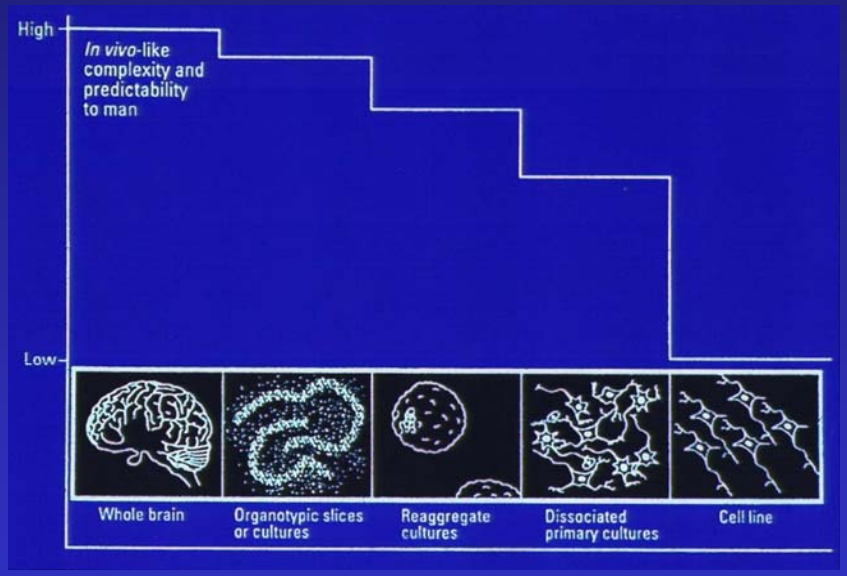
Tox testing in the 21st century



Collins et al., 2008, Science 319:906-907.

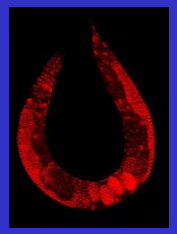
Alternative approaches to DNT:
Faster, more efficient and more humane

- ***In silico***
  - Quantitative structure-activity relationships (QSAR)
  - Computer modeling



- ***In vitro***
  - Cell lines
  - Primary cell cultures
  - **Human stem cells**
  - Organotypic cell cultures

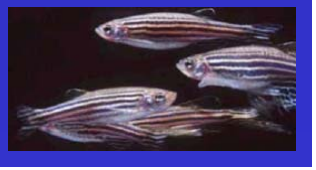
- **Non-mammalian systems-based models**
  - Nematode (*Caenorhabditis elegans*)
  - Fruit fly (*Drosophila melanogaster*)
  - Zebrafish (*Danio rerio*)



C. elegans

Drosophila

Zebrafish



In vitro models for DNT: Advantages

- **Reduces costs and animal use**
- **Facilitates screening of large numbers of chemicals *(high-throughput)***
- **Gene expression readily manipulated and monitored**
  - **Integrate molecular data with structural and functional changes**
  - **Incorporate relevant genetic polymorphisms**
- **Availability of human cells**

The Toxicological Paradigm:

or why scientists think in vitro models may be useful in predicting clinical outcomes

In vivo toxicity:

Exposure → Internal Dose → Biologically Effective Dose → Early Biological Effect (Molecular) → Altered Structure/ Function (Cell, Tissue, Organ) → Disease Dysfunction

In vitro toxicity:

Concentration → Biologically Effective Dose → Early Biological Effect → Altered Cellular Structure/ Function

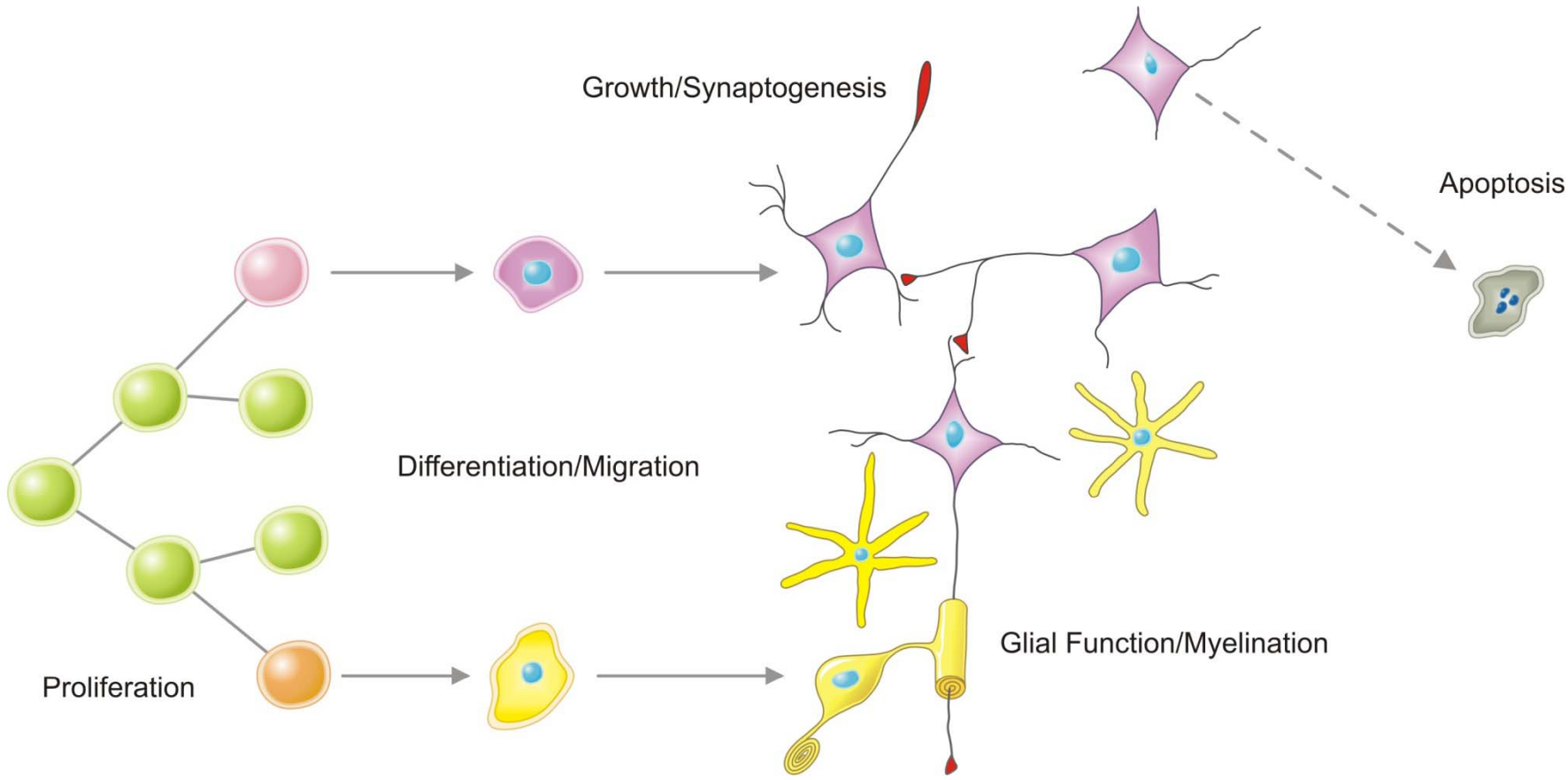
The most effective in vitro toxicity tests will be those that incorporate relevant biological (cellular and molecular) mechanisms

However,

The cellular and molecular mechanisms are not known for most toxicological outcomes in the developing nervous system

The Toxicological Paradigm and DNT

***In vitro* and alternative systems-based models replicate key evolutionarily conserved processes of neurodevelopment**



Schematic courtesy of Bill Mundy, U.S.E.P.A.

The Toxicological Paradigm:
Modulating factors that influence outcome

***In vivo* toxicity:**

Systemic influences (hormonal, metabolism, immune function)

Genetic susceptibility and effect modifier (diet, health, co-exposures, etc)

Exposure → Internal Dose → Biologically Effective Dose → Early Biological Effect (Molecular) → Altered Structure/ Function (Cell, Tissue, Organ) → Disease Dysfunction

Compensatory mechanisms ←

***In vitro* toxicity:**

Concentration → Biologically Effective Dose → Early Biological Effect → Altered Cellular Structure/ Function

Challenges of using *in vitro* models for DNT

- **Neurodevelopment is contextually driven**

  varies across brain regions
  dependent on developmental stage
  may differ between CNS and PNS
  requires cell-cell interactions
  influenced by genetic background

- **Assessing contributions of extraneural influences on developmental neurotoxicity**

  metabolism
  hormonal influences
  immunological function

- **Identifying the best model(s) to develop for DNT**

  predictive of in vivo effects

Major advantages of *in vitro* and alternative systems-based models for DNT

Replicate discrete stages of neurodevelopment

- *immediate biological relevance*
- *study multiple stages, multiple processes*

Relatively simple models

- *facilitates detection of subtle toxicant effects*

Gene expression readily manipulated and monitored

- *integrate molecular data with structural and functional changes*
- *incorporate relevant genetic polymorphisms to study gene X environment interactions*

DNT-relevant endpoints that can be readily assessed *in vitro* in addition to neurodevelopmental endpoints

- Cell viability/death
- Cell morphology
- Biochemical markers
- Neurotransmission
- Intracellular signaling
- Gene expression

Potential applications of *in vitro* DNT models for in risk assessment

- **Screening of large numbers of chemicals (high-throughput)**
- **Toxicodynamics (identifying mode of action)**
  - **Provide data for prioritization of chemicals for further targeted testing in animals (reduce number of animals used in testing)**
  - **Inform experimental design for animal testing (refine animal testing)**
  - **Provide data for developing and/or testing SAR (replace animal testing)**