U.S. Industry Perspective: DNT Testing Strategies Based on Alternative Assays

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The Human Element at Work.

Agenda

- Industry view of alternative assays
  - Why use alternative approaches?
  - Current use of alternative models
  - Integrating assay data with exposure
- *In vivo* approaches to evaluate Neurotoxicity/DNT
- Alternative DNT approaches and their challenges
- Characteristics of DNT Test Systems
- The Exposure-Effect Discontinuum
- Conclusions

Why Use of Alternative Approaches?

- *In silico*, *in vitro* and alternative *in vivo* models
- Industry supports alternative approaches to assess toxicity, including DNT:
  - Initial hazard characterization (early in development)
  - Prioritize testing based on bioactivities of potential concern
  - Select R&D candidate compounds
  - Test formulations
  - Used for read across
  - Targeted toxicity testing
  - Generate more complete toxicity evaluations
  - Evaluate potential MOA (mode of action)

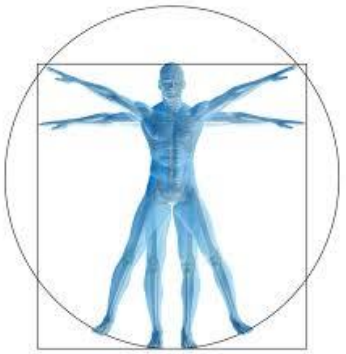
Current List of Alternative Models

Predictive Toxicology Approaches (Mammalian/Environmental)
Cheminformatics (*in silico* models) • QSAR • Analog ID & Read across • Metabolism modeling • Systemic exposure
Exposure Modeling • HTP • IVIVE
Biological Profiling (*in vitro* approaches) • Dermal/ocular corrosion and Irritation • Skin sensitization • Phototoxicity • Respiratory irritation • Mutagenicity • Endocrine Activity • DART • Toxicogenomics • Oxidative Stress • PBT • BCF • *In vitro* TK • Microplate acute ecotox screening

Alternative Models must be "fit for purpose" (e.g., are results for prioritization vs. regulatory submission?)

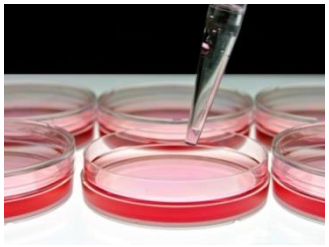
Integration: Assay Data + Exposure (IVIVE)

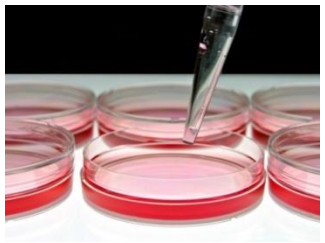
Predict exposure producing bioactive concentration of compound at the target site

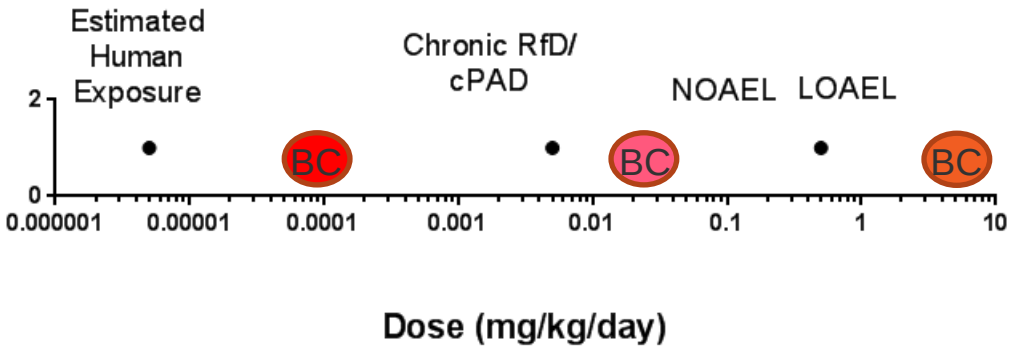


Rat *in vitro* data to predict *in vivo* exposure…

Human PBPK + *in vitro* data to predict human dosimetry







In vivo Approaches to Evaluate Neurotoxicity/DNT

- Testing requirements for industrial chemicals depend on tonnage produced (REACH), use/potential for exposure, NT signs, etc.

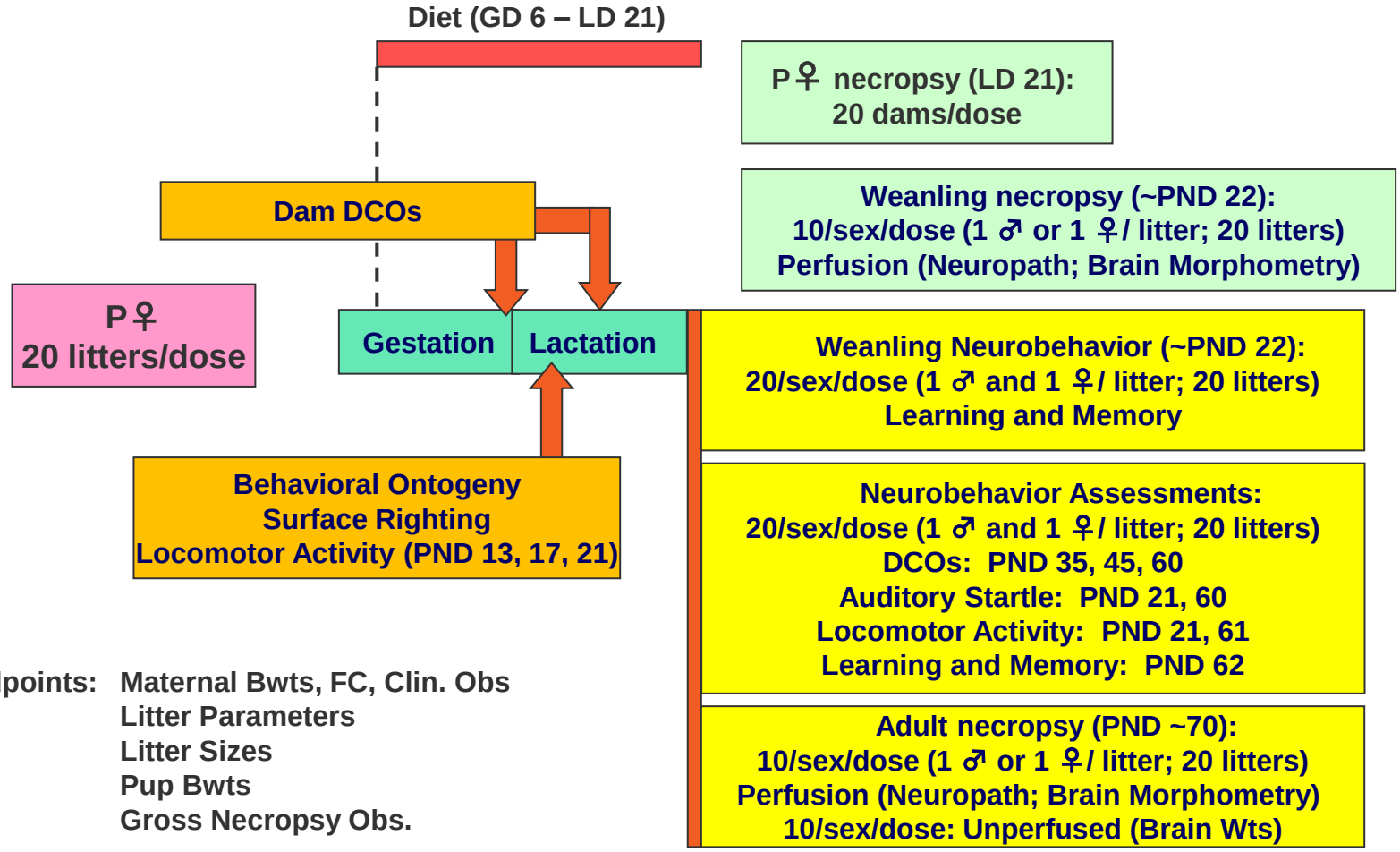
In vivo Approaches for Neurotoxicity and DNT

- Acute toxicity/neurotoxicity studies
- Repeat-dose toxicity/neurotoxicity studies (28-day, 90-day)
- Repro/devtl/repeat-dose screening assays (OECD 421/422)
- Developmental toxicity studies (OECD 414)
- Endocrine effects (e.g., thyroid – repeat-dose, OECD 421/422, pubertal assays, etc.)
- Two-generation (OECD 416) or EOGRTS (OECD 443)
- Developmental neurotoxicity study (OECD 426)

- Neurotoxicity target is unknown (need to detect a spectrum of effects)
  - Neurobehavioral assessments to evaluate integrated NS function
  - Neuropathology

Labor and Resource Intensive

DNT Study (OECD 426)

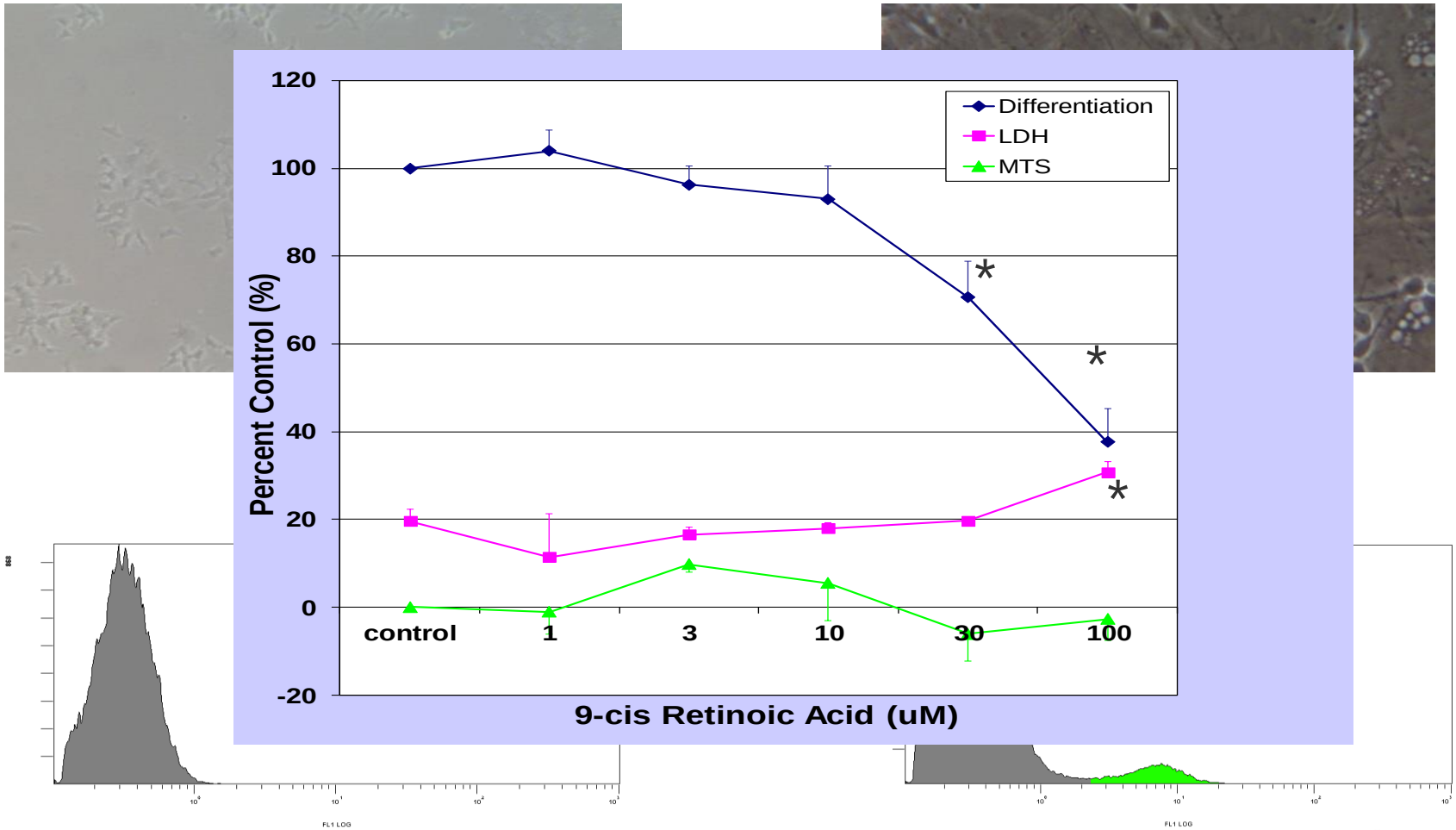


- Exposure to offspring confirmed with PK
- Retrospective analysis: DNT solely determines RfD ~5% (US EPA)

Alternative Assays to Detect DNT

- Assays should include effects specific for DNT (e.g., neuronal proliferation, migration, differentiation, myelination, etc.)
- Numerous alternative models in development:
  - Primary cells: neurons and glia from different brain regions
  - Neural stem (progenitor) cells
  - Cell lines: neuroblastoma, astrocytoma, glioma,
  - Organotypic (3D) co-cultures
  - Organisms: *C. elegans*, Zebrafish
- Detection methods:
  - Multi electrode arrays
  - Neurite outgrowth, Neurodegeneration, Cell proliferation, Apoptosis
  - Calcium flux
  - Synaptogenesis
  - Behavioral changes
- Need a battery approach/an integrated system that can detect multiple MOAs

Neuronal Differentiation with NT2 Cells



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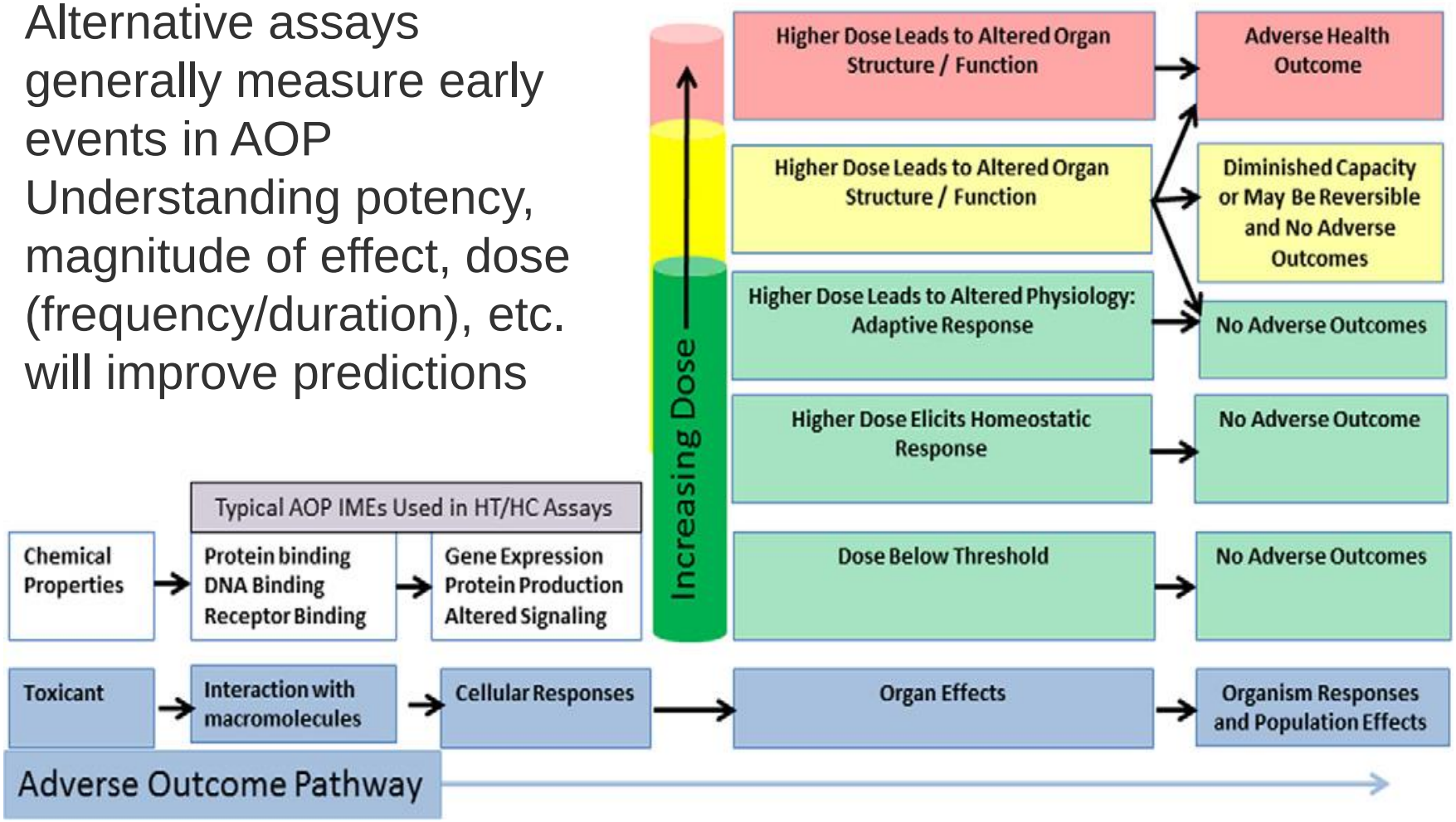
Characteristics of DNT Test Systems

- Bioprofiling…confidence is key!
  - Validity: relevance, reliability, sensitivity, specificity of assays
  - Regulatory agencies, regulated community and public must be confident
- Test systems should include:
  - Rationale and purpose for method
  - Relationship of test endpoint(s) to biological effect of interest
    - ID hazards relevant to human health (adverse effect that is biologically-plausible at relevant concentrations)
  - Detailed protocol
  - Chemical domains of applicability
  - Criteria for data interpretation (prediction model)
  - Assay limitations (e.g., in vitro metabolism/ADME, cell stress/cytotoxicity, non-specific effects, potency predictions)
  - Procedures to ascertain method performance (pos/neg controls for reproducibility; performance criteria, sensitivity; etc.)

The Exposure-Effect Discontinuum

- Alternative assays generally measure early events in AOP
- Understanding potency, magnitude of effect, dose (frequency/duration), etc. will improve predictions



Patlewicz et al., Reg. Toxicol. Pharmacol. 65:259, 2013.

Conclusions

- Alternative approaches will allow for more rapid screening and prioritization of compounds that show DNT potential
- Assays should be “fit for purpose” and results should be evaluated in the context of exposures
- Adequate evaluation of DNT potential may require a battery of assays
- Assay characterization will improve utility and scientific confidence to predict in vivo effects.
- Continued development of AOPs is needed with a focus on understanding key event relationships.