NAFTA Guidance for Developmental Neurotoxicity Interpretations: Background Clinical Signs/FOB

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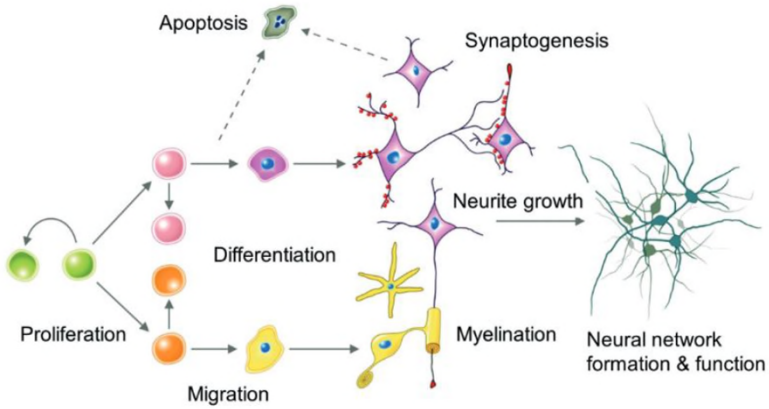
Nervous System Development

- Complicated sequence of neural cell development with specific temporal and spatial patterns
- Process is considered to be highly vulnerable to chemical exposures
- Critical periods of higher sensitivity, vary by chemicals
- Few known AOPs for developmental neurotoxicity, chemicals may act via multiple mechanisms



Public and Scientific Concerns about DNT

- Rising rates of neurodevelopmental disabilities and disease may be influenced by environmental chemical exposure
- To address concern, regulatory agencies developed comprehensive DNT testing guidelines
  - US EPA published guidelines (1998)
  - OECD modified EPA's guidelines (2007)
  - OECD created guidelines to streamline multiple developmental tests, decrease number of animals (2012)
- Even today, relatively few chemicals have been tested for developmental neurotoxicity

DNT Guideline Testing

	EPA 870.6300	OECD 426	OECD 443
Test species	Rat	Rat	Rat
Exposure	GD6 to weaning	GD6 to weaning	2 weeks pre-mating to weaning
Motor activity	Preweaning ontogeny and adult	Preweaning and adult	Preweaning and adult
Neuromotor ontogeny	None	Preweaning	None
Functional/Clinical observations	Throughout	Throughout	Adult
Auditory startle response	Weaning and adult	Weaning and adult	Weaning
Learning and memory	Weaning and adult	Weaning and adult	None
Neuropathology and morphology	Weaning and adult	Weaning and adult	Adult

In Vivo DNT Testing

- Exposure covers large part of developmental time frame
- Extensive testing of offspring from pre-weaning to adulthood
  - Maximizes chances of detecting behavioral and/or neurological changes
- Evaluates many aspects of nervous system function
- Issues with this broad-based approach
  - Specialized testing, require skilled personnel
  - Resource intensive: large investment of time, animals, and expense

Regulatory Uses of DNT Data

- Specific endpoints used as points of departure for risk assessment
- Inform uncertainty factors and labeling, such as FQPA in US, PCPA in Canada, hazard classification in EU.
- These uses can impact commercial interests such as approved uses, labeling, packaging, and import/export tolerances

Regulations across countries

- As more DNT studies have been conducted, inconsistencies have arisen in regulatory decision-making
- Differences in interpretations can lead to restricted or cancelled uses in some not all countries
- Significant impacts on international trade

	DNT Endpoint	Database UF
Regulator A	LOAEL lowest dose	3000
Regulator B	NOAEL lowest dose	100
Regulator C	NOAEL highest dose	300

Genesis of NAFTA Document

- Specific instances of conflicting pesticide assessments between US and Canada led to a decision to develop guiding principles for harmonization of data interpretation
  - Received funding from NAFTA for this effort
  - Relevant to regulators in all countries
- Collaboration of governmental experts so no commercial conflicts of interest

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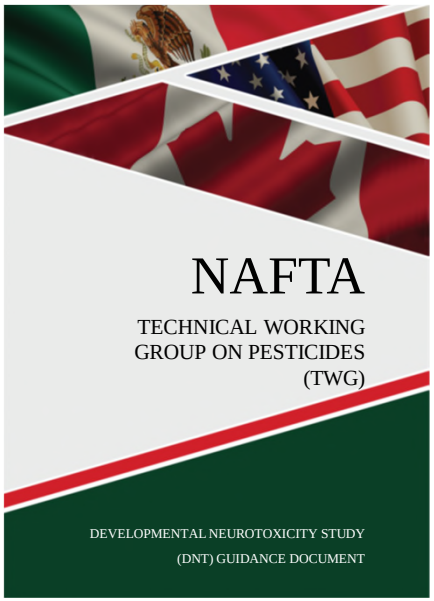
Kevin Crofton	Wayne Bowers
Kathleen Raffaele	
Mary Gilbert	



Goals of the NAFTA Document

- Improve understanding of DNT guideline studies
  - Appropriate conduct of behavioral tests
  - Biological significance of endpoints
- Increase consistency in interpretation and assessment of outcomes

- Focus on behavioral tests
  - Observations
  - Motor activity
  - Auditory startle response
  - Learning and memory
- Neuropathology/morphometrics not included
  - Several excellent peer-reviewed publications already available

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What Observations?

Functional observations

Expanded observations

Clinical observations

Functional observational battery

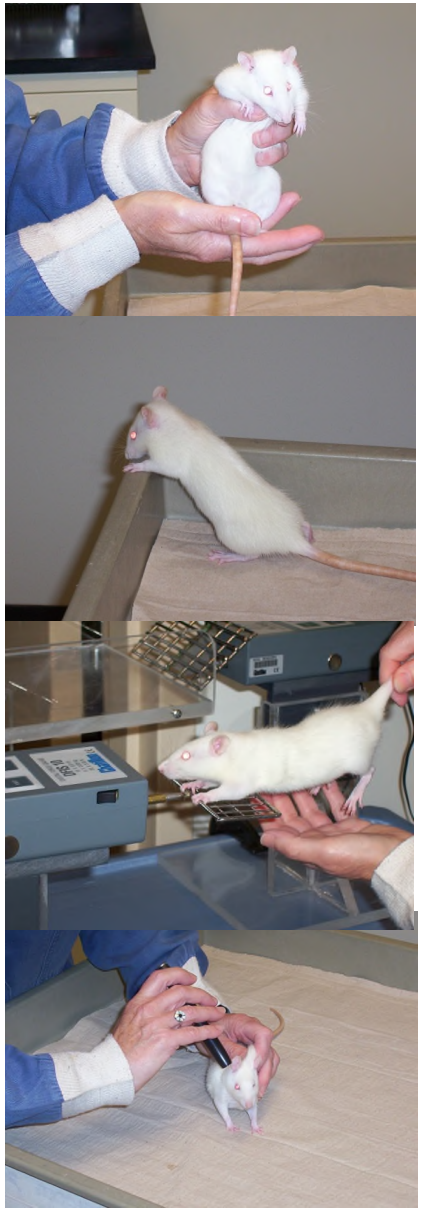
Detailed clinical observations

Cageside observations

FOB ≠ Clin Obs

FOB

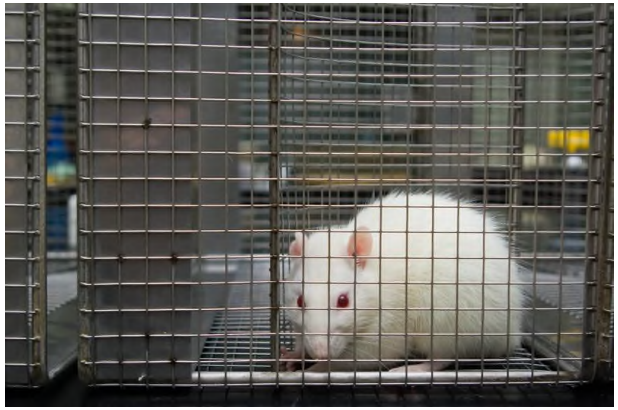
- Refers specifically to EPA* and OECD* guidelines for systematic and detailed evaluation of behavior
- Standardized series of tests
  - Takes place in home cage and open field
  - Includes observations and manipulative tests
  - Includes sensory and neuromotor tests
  - Many evaluations ranked or scored
- Requires trained and observers blind to treatment
- Based originally on Irwin screen to determine CNS actions of drugs in mice



*US EPA/OPPTS 870.6200 (1998); OECD 424 (1997)

Clinical Observations

- Clinical observations mostly not standardized
  - Similar to most other guidelines where overt animal health effects should be reported
  - Typically no detailed protocol
  - Often general list of signs that might be observed
    - “changes in skin and fur, eyes and mucous membranes, and respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern”
  - Few evaluations are ranked, mostly “normal” or “abnormal”
    - No definitions of “normal” or “abnormal”
    - Little value in detecting subtle changes in behavior
  - Evaluations mostly or always in home cage



Caution

- FOB was developed and validated with positive and negative controls to detect direct acute and/or chronic effects
  - Never been validated for detecting long-term effects, e.g., adult offspring following developmental exposure
  - Does not measure certain nervous system functions e.g., cognition, social interactions, affect behaviors
  - Labs have made their own modifications which may be very different from a standard FOB protocol
- Clin obs have never been formally validated

Requirements in DNT Guidelines

- FOB is only specifically required in OECD 443
  - DNT guidelines only require generalized list of observations
- Across testing labs, there is similarity in specific observations, but still very general
  - Autonomic
  - Motor
  - Convulsive
  - “Unusual or abnormal behaviors” catch-all
- Observe dams and offspring throughout study
  - Frequency varies in guidelines

Most labs conduct clin obs in DNT study even though they might call it FOB

How to Differentiate Various Clin Obs from FOB

Key Components of Protocol	FOB	Expanded Clin Obs	Clin Obs
Remove subject from cage and place on open field for observation	√	√	--
Observations ranked using explicit and standardized scales	√	√	--
Detailed protocol with explicit procedures	√	√ or --	--
Experimental design specifications, *e.g.*, counterbalancing, observer blind to treatment conditions	√	--	--
Non-observational measures, *e.g.*, grip strength, landing foot splay, body temperature	√	--	--

Important Information in Study Protocol and/or Report

- Look for
  - List of signs to be observed
  - Defined scoring criteria or explicit descriptions of “normal” and “abnormal”
  - Order and timing of testing
  - Observations made blind with respect to treatment
  - Training and experience of observer
  - Whether same observer used throughout
  - Whether same animal is tested each time, especially pups
  - Good experimental control
  - Accounting for age of subject, e.g., underdeveloped motor function in pups

Data Checks and Interpretations

- Control group
  - Look for variability in controls where expected
    - e.g., activity levels, reactivity, sensory responses vary across normal rats
  - Check for signs that are not expected
    - e.g., autonomic signs, tremors, convulsions
- Across dose groups
  - Look for patterns across time and/or dose
    - Tracking individual animals can be helpful
- Pattern of effects or consistency of functional responses
  - e.g., motor effects evidenced over several measures (activity, gait)
- Consider timing of observations with respect to chemical dosing

Example of “Good” Data

- Chemical (pesticide) that inhibits acetylcholinesterase
- Reported cholinergic clinical signs include salivation, lacrimation, lower activity, tremors in dams
- Data show dose- and time-related effects over 1st 3 weeks of dosing
- Conclude direct toxicity (concordance with known mechanism)

Tremors	Score*	Control	Low	Mid	High
Week 1	1 2 3	10 0 0	10 0 0	10 0 0	9 1 0
Week 2	1 2 3	10 0 0	10 0 0	8 2 0	7 3 0
Week 3	1 2 3	10 0 0	9 1 0	6 4 0	2 5 3

*Scoring
1=none
2=moderate
3=severe

Example of “Bad” Data

- Same chemical and dose/administration
- No reported effects on salivation, lacrimation, activity
- “Abnormal” for tremors at only at 3 weeks
  - Would at least expect activity to be impacted by tremors
- No clear pattern of effect (based on known mechanism)
- Low confidence in the results

Tremors	Finding	Control	Low	Mid	High
Week 1	Normal Abnormal	10 0	10 0	10 0	10 0
Week 2	Normal Abnormal	10 0	10 0	10 0	10 0
Week 3	Normal Abnormal	10 0	10 0	9 1	7 3

Example of Worst Data

"No clinical signs were observed"

There will always be a limited number of clinical signs in a study involving hundreds of animals!

Statistical Analyses: FOB or ClinObs

- Methods should be appropriate for type of data
  - Mostly non-parametric (e.g., ranks or scores) or binary (e.g., normal, abnormal)
- Within-subject repeated measures across time
  - Critical to know if same rat evaluated at all time points
- Biological vs statistical significance
  - Few findings of moderate or severe effects can be important even if not statistically significant
  - Transient effects should be considered treatment effect

Statistical Analyses: Overarching Issues*

- Methods should be appropriate for type of data
- Within-subject repeated measures
  - Analysis of each individual time point could lead to type 1 errors
- Sex as a factor
  - Only way to support conclusion of sex difference
- Litter is statistical unit
  - Littermates do not provide independent observations and must be accounted for
- Biological vs statistical significance
  - High variability leading to lack of significance should not negate obvious treatment effect

*The NAFTA report includes only a discussion of statistical issues specific to the types of behavioral tests used

Integrated Analysis of DNT Data

- Confidence in data
  - Appropriate statistical analyses
  - Well-conducted study with adequate reporting
- Patterns and consistency of effects for each endpoint
- Effective doses, severity, and patterns of effect across endpoints
- Maternal toxicity and offspring health and growth
- Available toxicity database
  - Known mechanism of action

Questions?

Thank you for your attention!