Regulatory Guidelines for Adult and Developmental Neurotoxicity Studies

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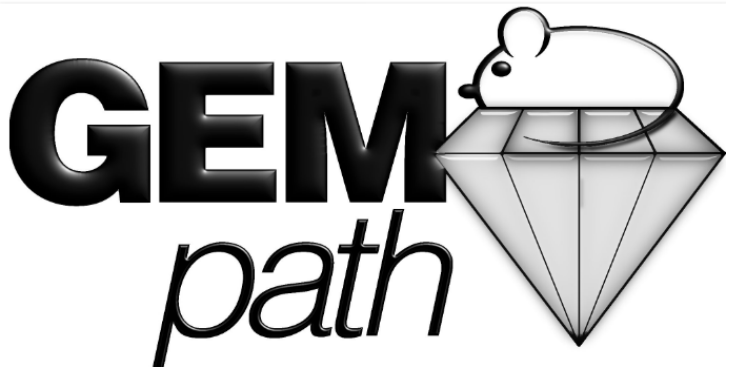
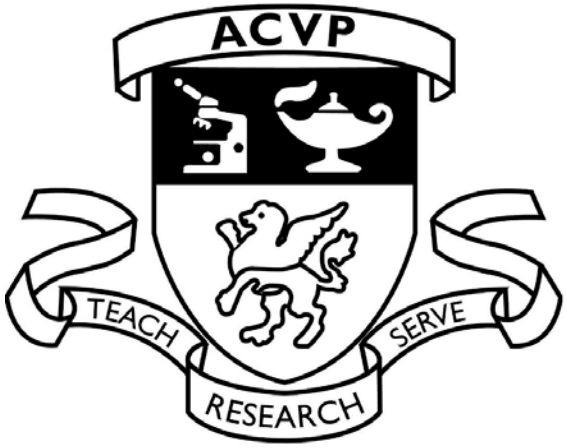


Brad's Main Professional Affiliations



Session Objectives

- **Review regulatory guidance for adult neurotoxicity studies**
- **Consider regulatory guidance for developmental neurotoxicity (DNT) studies**

Part I:

Key Global Agencies Regulating GLP-Type Neurotoxicity Studies

Regulatory Guidance Documents:
Neurotoxicity Studies – Medical Products

ICH (International Conference on Harmonization)

- **S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals [1997]**
- **S6(A1) Addendum to ICH S6: Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals [Step 3; 2009]**
- **S7A Safety Pharmacology Studies for Human Pharmaceuticals [2000]**
- **S9 Nonclinical Evaluation for Anticancer Pharmaceuticals [2009]**
- **M3(R2) Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals [2009]**

Regulatory Guidance Documents:
Neurotoxicity Studies – Medical Products

EMA (European Medicines Agency) – follows ICH guidelines

FDA (U.S. Food and Drug Administration) – multiple divisions

- **CBER (Center for Biologics Evaluation and Research)**
- **CDER (Center for Drug Evaluation and Research)**
- **CDRH (Center for Devices and Radiological Health)**
- **CFSAN (Center for Food Safety and Applied Nutrition) – *Redbook***
  - **IV: Guidelines for Toxicity Studies**
    - **Section C.10: Neurotoxicity Studies (2000)**
- **OOPD (Office of Orphan Products Development)**

Regulatory Guidance Documents:
Neurotoxicity Studies – Medical Products

OECD (Organisation for Economic Co-operation and Development)

- **Test No. 418:** Delayed Neurotoxicity of Organophosphorus Substances Following Acute Exposure [1995]
- **Test No. 419:** Delayed Neurotoxicity of Organophosphorus Substances: 28-day Repeated Dose Study [1995]
- **Test No. 424:** Neurotoxicity Study in Rodents [1997]
- **Test No. 426:** Developmental Neurotoxicity Study [2007]

Pharmaceuticals and Medical Devices Agency (PMDA [Japan])

– follows ICH guidelines

Regulatory Guidance Documents:
Neurotoxicity Studies – Chemicals

EPA (U.S. Environmental Protection Agency)

- Guidelines for Neurotoxicity Risk Assessment [1998]
- Title 40: Protection of Environment; Chapter I - Environmental Protection Agency
  - Part 79 – Registration of Fuels and Fuel Additives – § 79.66 and § 79.66
  - Part 158 – Data Requirements for Pesticides
    - Group E – Neurotoxicity Test Guidelines
      - 870.6100 Acute and 28-Day Delayed Neurotoxicity of Organophosphorus Substances [1998]
      - 870.6200 Neurotoxicity Screening Battery [1998]
      - 870.6300 Developmental Neurotoxicity Study [1998]
      - 870.6500 Schedule-Controlled Operant Behavior [1998]
      - 870.6850 Peripheral Nerve Function [1998]
      - 870.6855 Neurophysiology Sensory Evoked Potentials [1998]

Resource for On-line Access to Neurotoxicity Guidelines

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Compilation of International Regulatory Guidance Documents for Neuropathology Assessment During Nonclinical General Toxicity and Specialized Neurotoxicity Studies

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Abstract

Neuropathology analyses as end points during nonclinical efficacy and toxicity studies are challenging and require trained personnel and particular equipment to achieve optimal results. Accordingly, many regulatory agencies have produced explicit guidelines for designing and performing neuropathology assessments for nonclinical studies. This compilation of international regulatory guidance for toxicologic neuropathology end points represents a set of criteria recommended for general toxicity studies and specialized neurotoxicity studies that should facilitate the efforts of individuals who plan, perform, analyze, and report neuropathology evaluations in nonclinical toxicity studies.

Keywords: guidance; guideline; Internet; neuropathology; neurotoxicology; regulatory; toxicologic pathology; regulatory affairs.

Part II:

Regulatory Guidance for GLP-Type Adult Neurotoxicity Studies

Aims of Adult Neurotoxicity Testing

- **Define target regions and cells**
- **Identify classes of lesions**
  - ***Macroscopic findings***
    - **Changes in brain size – organ weight**
    - **Discoloration – necrosis (“malacia”) or hemorrhage**
  - ***Microscopic alterations***
    - **Cell degeneration / death (especially neuronal)**
    - **Glial hyperplasia (tissue response to neural injury)**
    - **Vacuolation (axonal degeneration or demyelination)**
- **Determine whether or not lesions are reversible**
- **Understand pathogenic mechanisms**

Key Guidelines for Adult Neurotoxicity Testing

- **OECD (Organisation for Economic Co-operation and Development) (1997). Guideline for the Testing of Chemicals: 424, *Neurotoxicity Study in Rodents***

- **EPA (U.S. Environmental Protection Agency) (1998). Health Effects Test Guidelines: OPPTS 870.6200, *Neurotoxicity Screening Battery***

Guidance on Designing Adult Neurotoxicity Tests in Rodents

- **Subject – rat (other species if warranted)**
  - **Males and females**
  - **Young adults (42 days at first exposure, not older than 54 days)**

- **Exposure – 28, 90, or 365 days**
  - **Dose groups – 4 (or 5 [to prevent large intervals])**
    - **High Dose: significant non-lethal toxicity (ideally neural)**
    - **Intermediate Dose: mid-way between high and low doses**
    - **Low Dose: minimal or no clinical toxicity**
    - **Controls:**
      - **Negative (vehicle-treated), concurrent**
      - **Positive, ideally concurrent but historical acceptable**
  - **Group size**
    - **Behavioral studies (multiple time points) – 10 per sex per group**
    - **Neuropathology (terminal) = 5 per sex per group**
  - **Route – defined by route of human exposure, but oral preferred**

Guidance on Functional Endpoints for Adult Neurotoxicity Tests in Rodents

- **Timing – depends on study length (best defined in OECD)**
  - **Acute = study days 7, 14, 28 (terminal)**
  - **Subacute = days 7, 14, 28, 56, 90 (terminal)**
  - **Chronic = days 7, 14, 28, 56, 90, 180, 365 (terminal)**
- **Test stratification**
  - **Design: "blinded" evaluation, ideally by one person (or with data to show concordance of multi-person observations)**
  - **Clinical signs / functional observational battery (FOB) – all animals**
  - **Behavioral testing – "behavioral" cohort only – home cage + open field**
    - **Motor Activity (by automated recording)**
    - **Sensory ability – auditory, proprioceptive, visual**
    - **Strength (forelimb / hind limb grip)**

Guidance on Tissue Processing for Adult Neurotoxicity Tests in Rodents

- **Fixation**
  - **Procedure – perfusion**
  - **Preservative – “appropriate aldehyde”**
- **Embedding**
  - **CNS – paraffin**
  - **PNS – plastic (routine or if signs suggest nerve is affected)**
- **Stains**
  - **H&E**
  - **Special procedures (if warranted)**
    - **Neuronal / axonal degeneration (silver stain)**
    - **Tissue response to neural injury (anti-glial fibrillary acidic protein [GFAP])**

Guidance on Microscopic Analysis for Adult Neurotoxicity Tests in Rodents

- **Strategy – stepwise approach**
  - **High-dose and control tissues are assessed first**
  - **Other dose groups added sequentially (intermediate, low if needed)**
- **Assessment paradigm**
  - **Analysis should include CNS and PNS samples from:**
    - **"Representative" or "all major" regions**
    - **Sites "known to be sensitive" to neurotoxic insult**
    - **Key effector organs (eye, skeletal muscle ["calf"])**
  - **Evaluation**
    - **Initial qualitative analysis – "unblinded"**
    - **Follow-up semi-quantitative analysis – to define dose-response**
      - **Nature of review: "blinded" and "randomized," for sites with lesions**
      - **Rationale: correlate lesion frequency / severity with test article dose**
      - **Statistical analysis of histopathology data is required**

Dedicated Adult Neurotoxicity Studies:
Design Guidance for Neuropathology Endpoints

Guidance Title	Guideline Number	Group Size	Control Groups
Acute and 28-Day Delayed Neurotoxicity of OPs	EPA 870.6100	6 hens	Vehicle + Positive
Acute Delayed Neurotoxicity of OPs	OECD 418	6 hens	Vehicle + Positive
28-Day Delayed Neurotoxicity of OPs	OECD 419	6 hens	Vehicle
Neurotoxicity Testing Battery	EPA 870.6200	5 rats/sex	Vehicle + Historical Positive
Neurotoxicity Testing Battery	OECD 424	5 rats/sex	Vehicle + Historical Positive
Neurotoxicity Testing Battery	FDA Redbook 2000 IV.C.10	Sufficient for valid test	Suitable

Dedicated Adult Neurotoxicity Studies:
Brain Sampling for Neuropathology

Guideline Title	Guideline Number	MR	OB	Ce	BG	Hi	T	Hy	M	Cb	P	MO
Acute and 28-Day Delayed Neurotoxicity of OPs	EPA 870.6100											X
Acute Delayed Neurotoxicity of OPs	OECD 418									X		X
28-Day Delayed Neurotoxicity of OPs	OECD 419									X		X
Neurotoxicity Screening Battery	EPA 870.6200	X		X						X	X	
Neurotoxicity Screening Battery	OECD 424	X		X		X	X	X	X	X	X	X
Neurotoxicity Screening Battery	FDA Redbook 2000 IV.C.10	X										

Brain region abbreviations: MR = "multiple representative" samples for all brain regions, OB = olfactory bulb, Ce = cerebrum, BG = basal ganglia, Hi = hippocampus, T = thalamus, Hy = hypothalamus, M = midbrain, Cb = cerebellum, P = pons, MO = medulla oblongata

Dedicated Adult Neurotoxicity Studies:
Spinal Cord / PNS Sampling for Neuropathology

Guideline Title	Guideline Number	Spinal Cord				Nerve Roots		Peripheral Nerves					
		N	C	T	L	DRG	SRN	N	S/T	SP	TP	TD	TB
Acute and 28-Day Delayed Neurotoxicity of OPs	EPA 870.6100		X	X	X						Bi		Bi
Acute Delayed Neurotoxicity of OPs	OECD 418		X	X	X						Bi		Bi
28-Day Delayed Neurotoxicity of OPs	OECD 419		X	X	X						Bi		Bi
Neurotoxicity Screening Battery	EPA 870.6200	X							X				
Neurotoxicity Screening Battery	OECD 424		X		X	X	X			X	Bi	X	
Neurotoxicity Screening Battery	FDA Redbook 2000 IV.C.10		X	X	X				X				

Region abbreviations: N = "not otherwise specified", C = cervical, T = thoracic, L = lumbar, DRG = dorsal root ganglion, SRN = spinal nerve roots, S/T = sciatic or tibial (location not specified), SP = proximal sciatic, TP = proximal tibial, TD = distal tibial, TB = branch of tibial, Bi = bilateral

General Toxicity Studies:
Sampling Guidance for Neuropathology Endpoints

Guideline	Guideline Number	CNS	Brain				
		MR	MR	Ce	Cb	P	MO
90-Day Dermal Toxicity	OECD 411		X	X	X	X	X
Repeated Dose 28-Day Oral Toxicity in Rodents	EPA 870.3050		X	X	X	X	
Repeated Dose 28-Day Oral Toxicity in Rodents	OECD 407		X	X	X	X	
All Other Guidelines except for FDA			X	X	X	X	X
FDA Guidances		X	X				

Brain region abbreviations: MR = "multiple representative" samples for all brain regions, Ce = cerebrum, Cb = cerebellum, P = pons, MO = medulla oblongata

General Toxicity Studies:
Sampling Guidance for Neuropathology Endpoints

Guideline	Guideline Number	NOS	Spinal Cord				Nerve
		MR	NOS	C	T	L	Sciatic or Tibial
90-Day Dermal Toxicity	OECD 411		X				X
Repeated Dose 28-Day Oral Toxicity in Rodents	EPA 870.3050		X				X
Repeated Dose 28-Day Oral Toxicity in Rodents	OECD 407		X				X
All Other Guidelines except for FDA				X	X	X	X
FDA Guidances		X		X	X	X	X

Regional abbreviations: N = "not otherwise specified", MR = "multiple representative" samples, C = cervical, T = thoracic, L = lumbar

Part III:

Regulatory Guidance for GLP-type Developmental Neurotoxicity (DNT) Studies

Aims of DNT Testing

- **Define target regions and cells – especially**
  - Cerebral cortex (associative, motor, sensory)
  - Hippocampus (learning, memory)
  - Cerebellum (motor)

  } Robust neuronal differentiation as well as synapse and transmitter production

- **Identify classes of lesions**
  - *Macroscopic findings*
    - Changes in region size or shape – asymmetry, hydrocephalus
    - Missing or new structures
  - *Microscopic alterations*
    - Cell degeneration / death (less common than in adults)
    - Cytoarchitectural anomalies – aberrant differentiation
    - Pattern disruptions – ectopia, migratory defects
    - Persistence of transitory structures - dysgenesis

Key Guidelines for Rodent Developmental Neurotoxicity Testing

- **OECD (Organisation for Economic Co-operation and Development) (2007). Guideline for the Testing of Chemicals: 426, *Developmental Neurotoxicity Study***

- **EPA (U.S. Environmental Protection Agency) (1998). Health Effects Test Guidelines: OPPTS 870.6300, *Developmental Neurotoxicity Study***

Some regulators consider OECD guidance to be the better approach (*Environ Health Perspect* 117: 17-25, 2009) … where "better" means "more flexible."

Guidance on Designing Rodent Developmental Neurotoxicity Tests

- **Subject – rat (common strains, not F/344)**
- **Exposure (maternal)**
  - **Groups – recommended N = 20 litters/group**
    - **High Dose: known toxicity to offspring or dam (slight)**
    - **Intermediate Dose: mid-way between high and low doses**
    - **Low Dose: no toxicity to offspring or dam**
    - **Control: negative (sham- or vehicle-treated), concurrent**
  - **Route – PO preferred, but other routes when relevant**
  - **Schedule – typically E6 to P10 (EPA) or P21 (OECD) – not on P0**
- **Sampling**
  - **Separate cohorts for functional tests and neuropathology**
  - **Culling – at random, not just removal of runts**
    - **reduce on P4 to 4 (EPA) or 4-6 (OECD) / sex / litter**
    - **discard litters if < 7 pups**
  - **N = 10 / sex / group / test; 1 male or 1 female per litter**

Guidance on Endpoints for Rodent Developmental Neurotoxicity Tests

- **Functional Deficits**
  - **Clinical Signs – P 4, 11, 21, 35, 45, 60 (EPA) or 60-70 (OECD)**
  - **Behavioral Ontogeny (2) – motor activity, negative geotaxis, righting**
  - **Developmental Landmarks – 13, 17, 21, ~30, ~45, 60**
  - **Acoustic Startle – P ~21, 60**
  - **Motor Activity – P 13, 17, 21, 60 ± 2 (EPA) or 60-70 (OECD)**
  - **Motor and Sensory Function – P ~21, 60-70**
  - **Learning and Memory – P ~21, 60 (EPA) or 60-70 (OECD)**
- **Neuropathology**
  - **Juvenile (P11) – brains weights + neuropathology**
  - **Weanling (P22) – brain weights + neuropathology**
  - **Young adult (P60 or 60-70) – brain weights + neuropathology**

Fixation of the Developing Nervous System

- **Immersion – suitable for P11 (EPA) or P11-22 (OECD)**
  - **Advantages: simple and rapid for modestly trained personnel**
  - **Disadvantages: unfixed neural tissues may be damaged at necropsy, fixation of deep brain regions may be inconsistent**
- **Perfusion – required for adult (P60-70)**
  - **Advantages: uniform fixation of neural tissues (CNS and PNS), post-fixation *in situ* reduces handling-related artifacts**
  - **Disadvantages: low-throughput, special equipment and training**
- **Fixative**
  - **EPA – an appropriate aldehyde**
  - **OECD – an appropriate fixative**
  - **Preferred: neutral buffered 10% formalin (3.7% formaldehyde)**
  - **Other: Bouin's solution, Karnovsky's fixative, paraformaldehyde**

Rodent Developmental Neuropathology Macroscopic Assessment

- **Euthanasia**
  - **P11 or P22 (EPA) = carbon dioxide**
  - **P11-22 (OECD) = a humane method**
  - **P60-70 = anesthesia suitable for perfusion fixation**
- **Central nervous system**
  - **Removal – brain**
  - **External examination (minimal handling to avoid artifacts)**
  - **Weight – brain (10 / sex / group)**
- **Peripheral nervous system (OECD = assess at P60-70)**
  - **Removal**
  - **External exam – usually cursory since few gross lesions**

Rodent Developmental Neuropathology Histological Processing

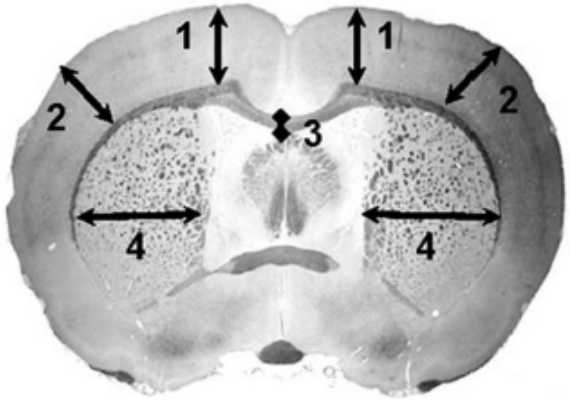
- **Processing Conditions (6 / sex / group)**
  - **Embedding: paraffin acceptable, plastic preferred**
  - **Section selection:**

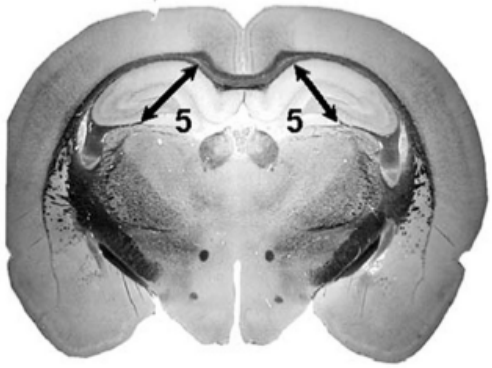
Identification of internal anatomic landmarks in coronal sections of brain floating on a water bath

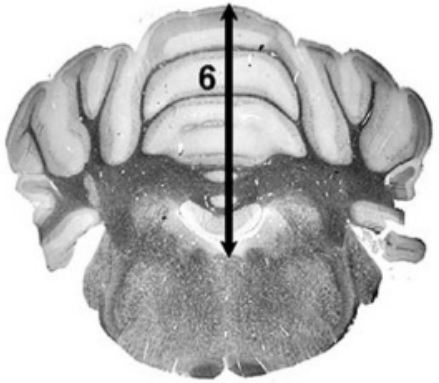
- **Staining**
  - **General stain: hematoxylin and eosin**
  - **Special stains: if warranted by professional judgment (EPA)**
    - **Cell-specific: cresyl violet (neurons) + Luxol fast blue (myelin)**
    - **Neuronal integrity: Bielschowsky's, Bodian's**
    - **Glial reaction: anti-GFAP – mainly P60**

Rodent Developmental Neuropathology Guidelines = Histomorphometry

- **Linear dimensions of major structures = minimum**
- **Sites – neocortex, hippocampus, cerebellum (EPA)**







1. Cerebral Cortex thickness (frontal)
2. Cerebral cortex thickness (parietal)
3. Corpus Callosum thickness
4. Striatum width
5. Hippocampus thickness
6. Cerebellum height

Toxicol Pathol 34: 296-313, 2006

Neuropathology Practices in Regulatory Agency Guidelines

- **General toxicity studies (and diagnostic cases)**
  - **Multiple "representative" sections – cerebrum, cerebellum, brainstem, spinal cord (± at specific levels), nerve**
  - **Immersion fixation, paraffin embedding**
  - **Special neurohistological procedures if needed**
- **Dedicated neurotoxicity studies (product testing)**
  - **Multiple "representative" sections**
    - **Adult – more regions required by OECD than U.S. EPA**
    - **Developmental – more regions specified than for adult**
  - **Perfusion fixation, sometimes plastic embedding (nerve)**
  - **Special neurohistological procedures necessary**
  - **Added structures – dorsal root ganglia, more nerves**