Juvenile Rat Toxicity Study: Science, Logistics and Issues for Pathologists.

STP-I DERMAL JUVENILE AND IMMUNE SYSTEMS

26-28 OCTOBER 2018 THE WESTIN MINDSPACE HYDERABAD INDIA

Friday, 26 October, 2018; 3:50 pm – 4:35 pm

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Juvenile Animal Toxicology Studies

- **Juvenile animal studies are not new and have been performed in the past for classes with pediatric use (e.g., antibiotics)**
- **Not every pediatric drug development requires juvenile animal studies!**

Juvenile Toxicity Studies Pediatric Population and Animal Models

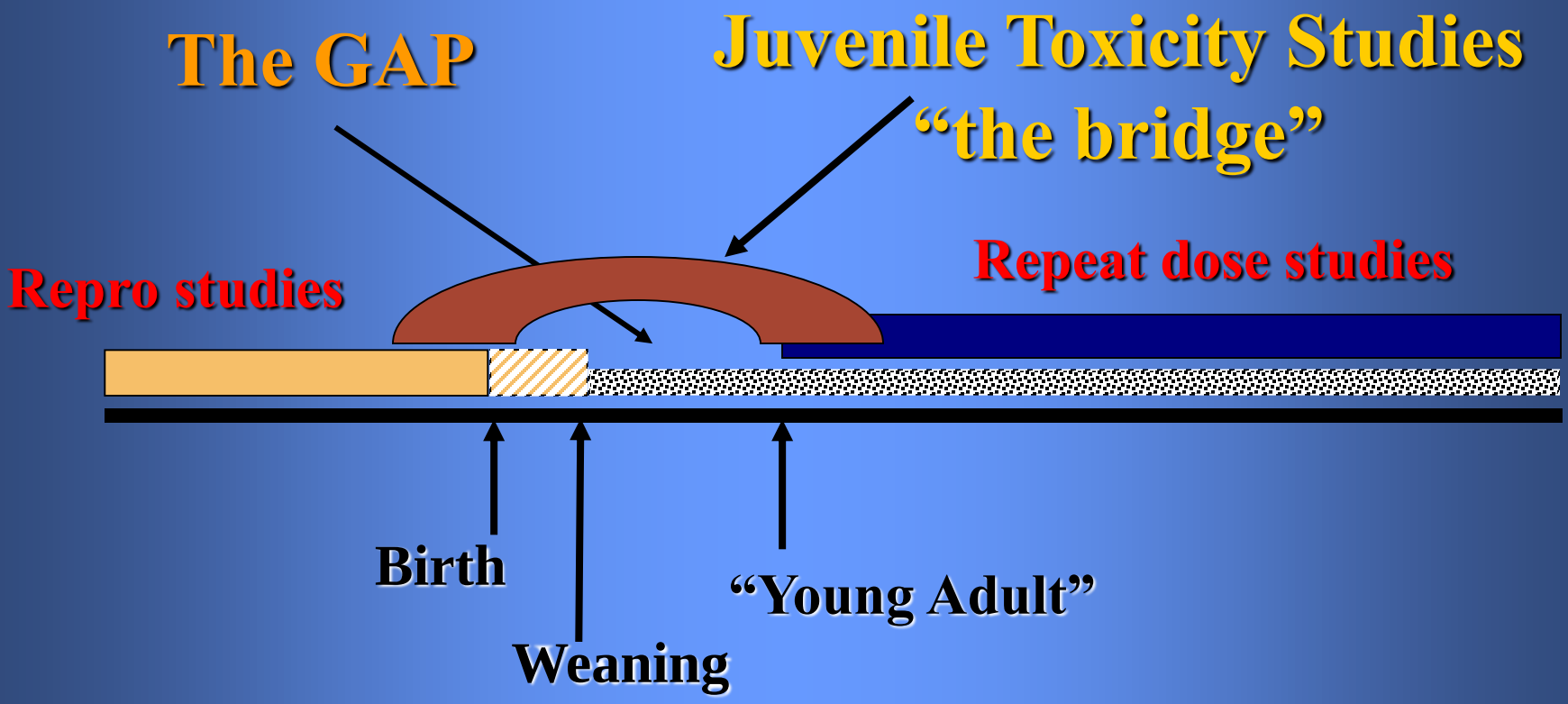
Children are not small adults.

Rats are not humans

Each pediatric age group is different

Bridging the GAP

Juvenile Toxicity Studies aim at bridging potential data-gaps between the pre- & postnatal toxicity studies (Seg III; offspring potentially exposed until weaning) and the repeat-dose toxicity studies (in young adult animals).



Juvenile Toxicology Is Complicated

- **Yes, even more complicated than adult Reproductive Toxicology**
- **Conceptually**
  - **Dynamic Anatomy & Physiology**
  - **Interdependence of endpoints**
  - **Statistics**
    - **Litter based**
    - **Historical Control Data is important**
  - **Neurobehavior (potential for latency)**
  - **Myriad modes of action**

Juvenile Toxicology Is Complicated

- **Logistically**
  - **Size/design of studies**
  - **Number and diversity of endpoints**
  - **Difficulty of scoring endpoints**
    - **direct effects**
    - **latent effects**
- ***Decreasing number of University training programs and therefore an ever decreasing pool of people skilled in these evaluations***
- ***On-the-Job training***

What Does A Study Director Need To Know to Effectively Design a Juvenile Toxicity Study?

- Indication and Intended Pediatric Population
- Gap Assessment
- Sensitive Species (1 or more) - Justification

  Toxicity and Target organs (Endpoints desired)

  TK data (ADME)/ Metabolic profile

- Mechanism of Action

  Receptor Ligand Binding Assay results/SAR information

- Route of Administration
- Available DART /General Toxicity Study data, including previous Juvenile Toxicity Data

Drug Effects on Growth & Development

- Development is a continuous process
- Structural/functional maturational differences affect drug safety
- Postnatal toxicity more likely in tissues undergoing postnatal development

Organ System	Maturation
• Neurologic	Adolescent-Adult
• Reproductive	Adolescent/puberty
• Pulmonary (alveoli)	Infant (1-2 years)
• Renal (function)	Infant (1 year)
• Skeletal	Adolescent-Adult
• Immune (IgG, IgA)	Infant-Child (5, 12 years)

Large Molecule Biotechnology-Derived Pharmaceutical (Biopharmaceuticals)

- Diverse group of therapeutics derived from biological sources or complex biotechnological processes
  - Examples: monoclonal antibodies, peptides, vaccines, allergen extracts, antitoxins and blood-derived products
- Specific challenges involved in testing
  - Diverse structural and biological properties
  - Species specificity
  - Potential for immunogenicity
- International Conference on Harmonization (ICH) S6 Guidance "Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals"
  - Flexible, case-by-case, science-based approach
  - Does not address juvenile toxicity assessments

Immunogenicity

- Development of Antidrug Antibodies
  - *Does not reliably correlate or predict immunogenicity in humans*
  - Can have significant impact in preclinical studies
    - Often reduce exposure in animals after repeated dosing
    - May neutralize the activity of the biopharmaceutical
    - May result in secondary toxicities related to immune complex formation or deposition

Principles For Postnatal/Juvenile Study Designs and Data Interpretation

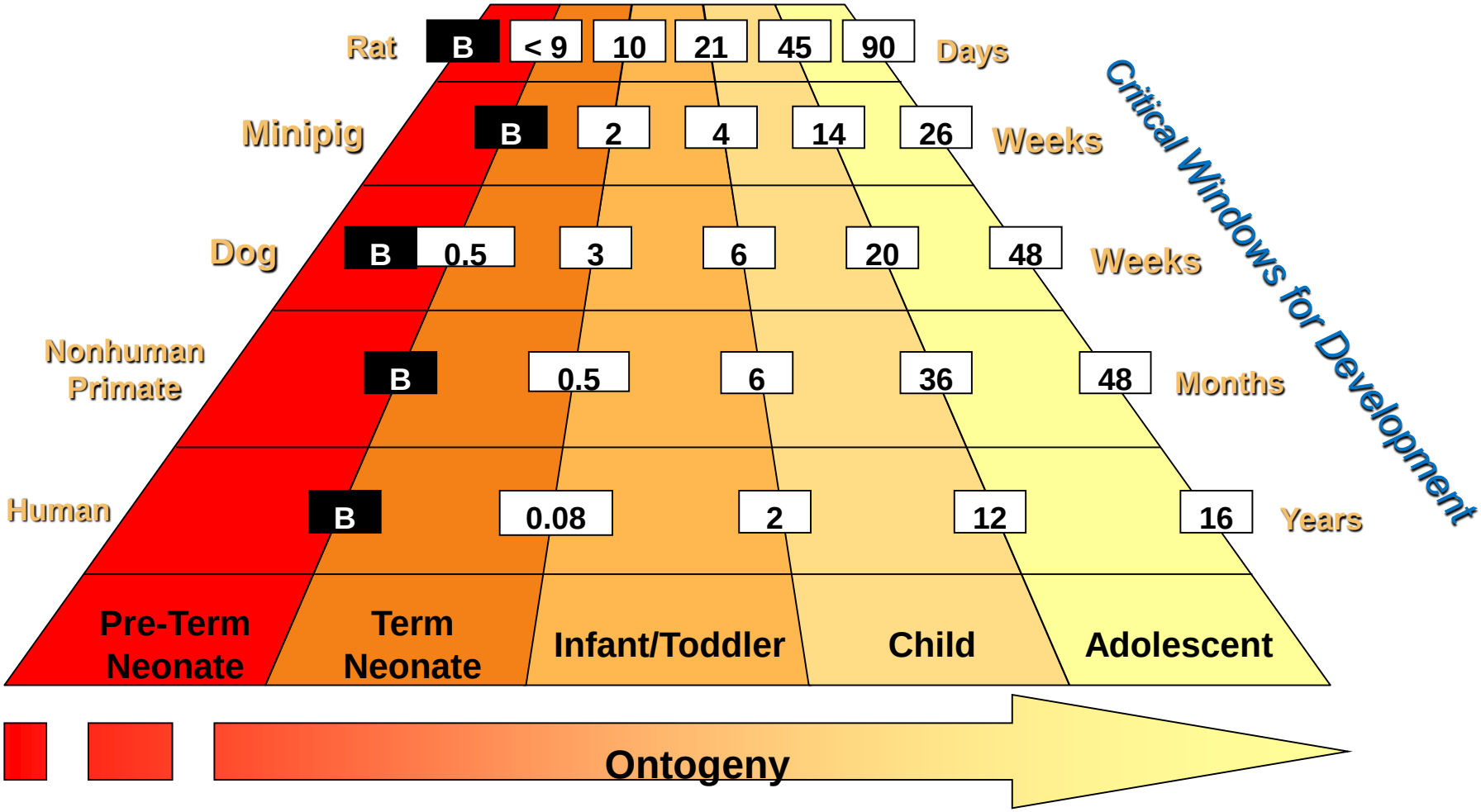
- Remember that the Litter is the largest contributor to physical and behavioral variability in rodents and actually becomes more so as animals age (CBTS results).
- Study design determines the conclusions that may be drawn from the data (=Successful Study)
- Sample size may vary based on study design complexity and logistical / technical issues
- Know your species - patterns are important

Litter Composition

- Within-Litter Design - Each litter has all Treatment Groups
- Split Litter Design - Each litter has some of the Treatment Groups
- Between-Litter Design - Each litter has the same Treatment Group
- Fostering Design
  - Each litter is composed of pups from other litters without using any siblings
  - All pups within new litter receive the same treatment
- One Pup per Sex per Litter Design

Each Method has its advantages and disadvantages (logistically, cross-contamination possibility, IACUC issues, and statistically)

Comparative Age Categories based on Overall CNS and Reproductive Development



Buelke-Sam, 2011

Semple

Rodents

- **Advantages**
  - Most frequently used for juvenile testing
  - Extensive experience/historical data across labs
  - Can test full span of postnatal development
  - Ability to procure appropriate numbers of animals, even for early age assessments
  - Wide range of tests available
    - Neurobehavioral
- **Disadvantages**
  - May not be pharmacologically relevant
  - small size limits routes of administration
  - Potential immunogenic response
  - Small size limits ability to collect multiple biologic specimens

Earliest Starting Day Based on Dosing Routes

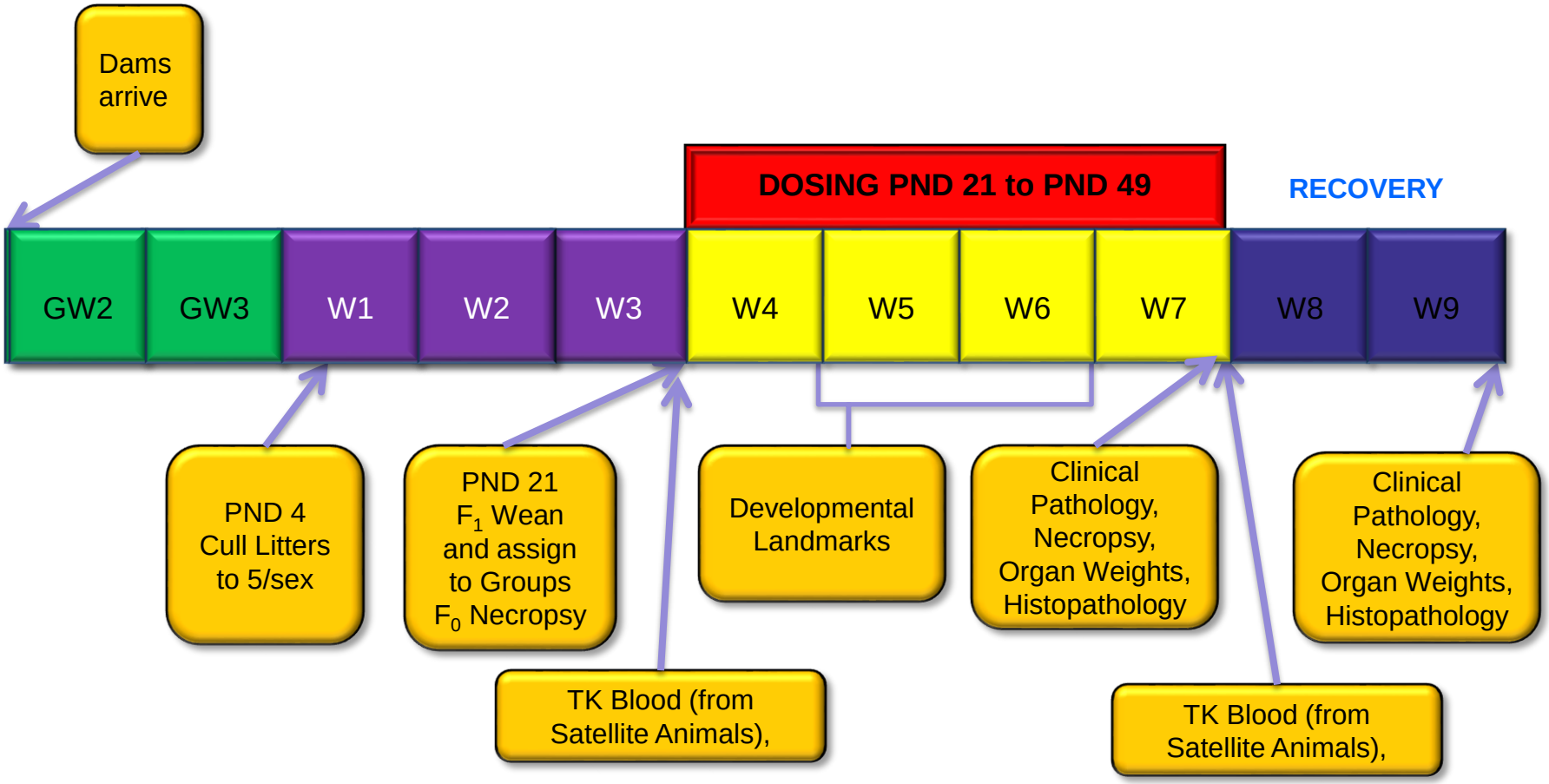
	Rat	Mice	Rabbit	Dog	Minipig
Oral gavage	PND 1	PND 4	PND 14	PND 1	PND 1
Subcutaneous	PND 1	PND 1	PND 6	PND 1	PND 1
Intramuscular	PND 1	PND 1	PND 6	PND 1	PND 1
IV bolus (repeated)	PND 4	PND 7	PND 6	PND 1	PND 7†
IV infusion	PND 21	PND 21	PND 28	PND 56	PND 7†
Inhalation (Whole Body)	PND 4	PND 4	PND 6	PND 10	PND 2-7
Inhalation (Nose only)	PND 21	PND 21	PND 28	PND 4**	PND 4**
Dermal *	PND 21	PND 21	PND 28	PND 42	PND 28

†= difficult because there are no easily accessible veins (Vascular implant port can be surgically installed after PND 7)

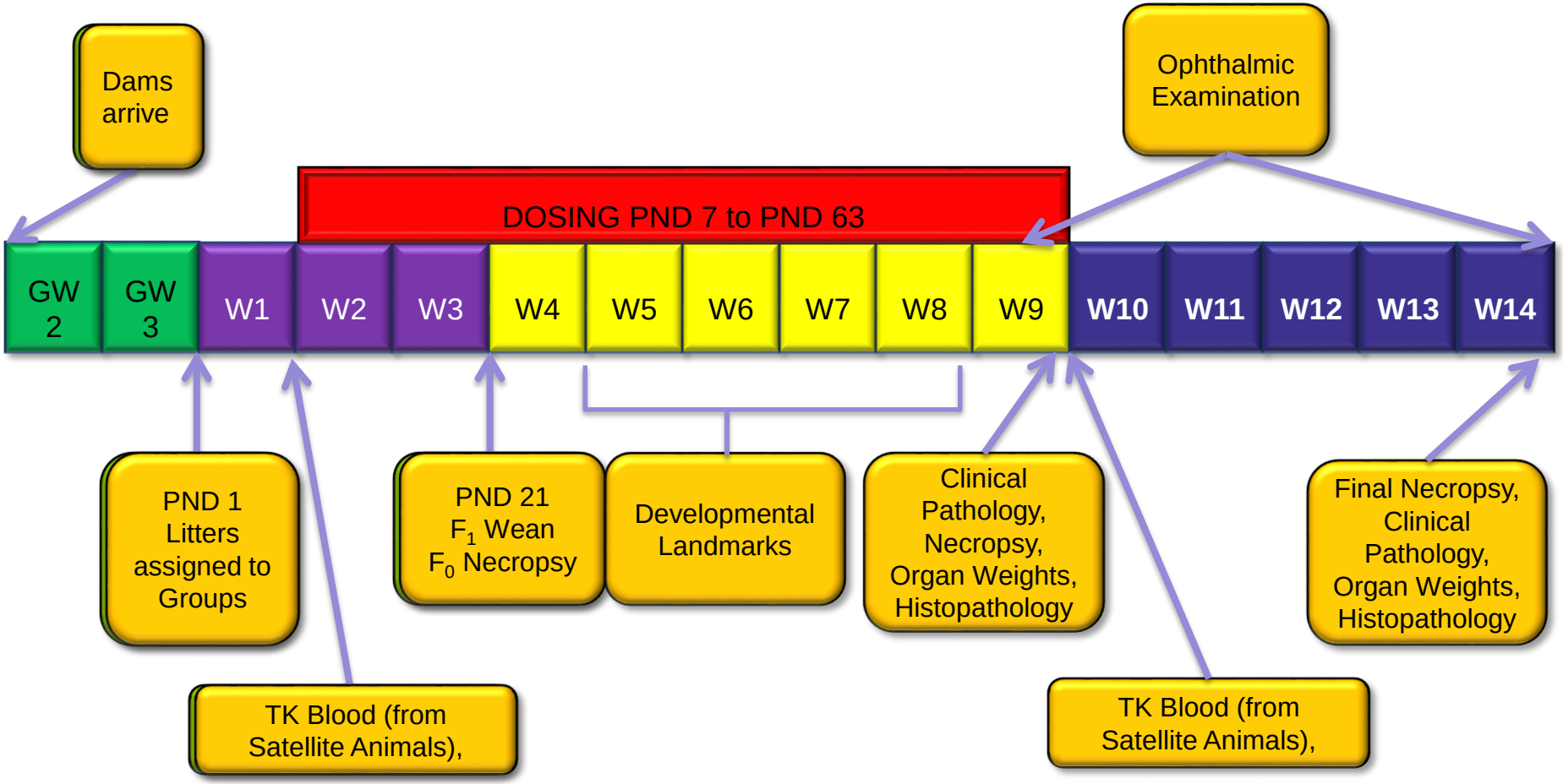
- = not recommended in pre-weaning animals
- ** = masks can be fit as young as PND 4 but restraint issues arise

Parker (2014),
Hoberman and Lewis (2010)
Luc De Schaepdrijver (2009)

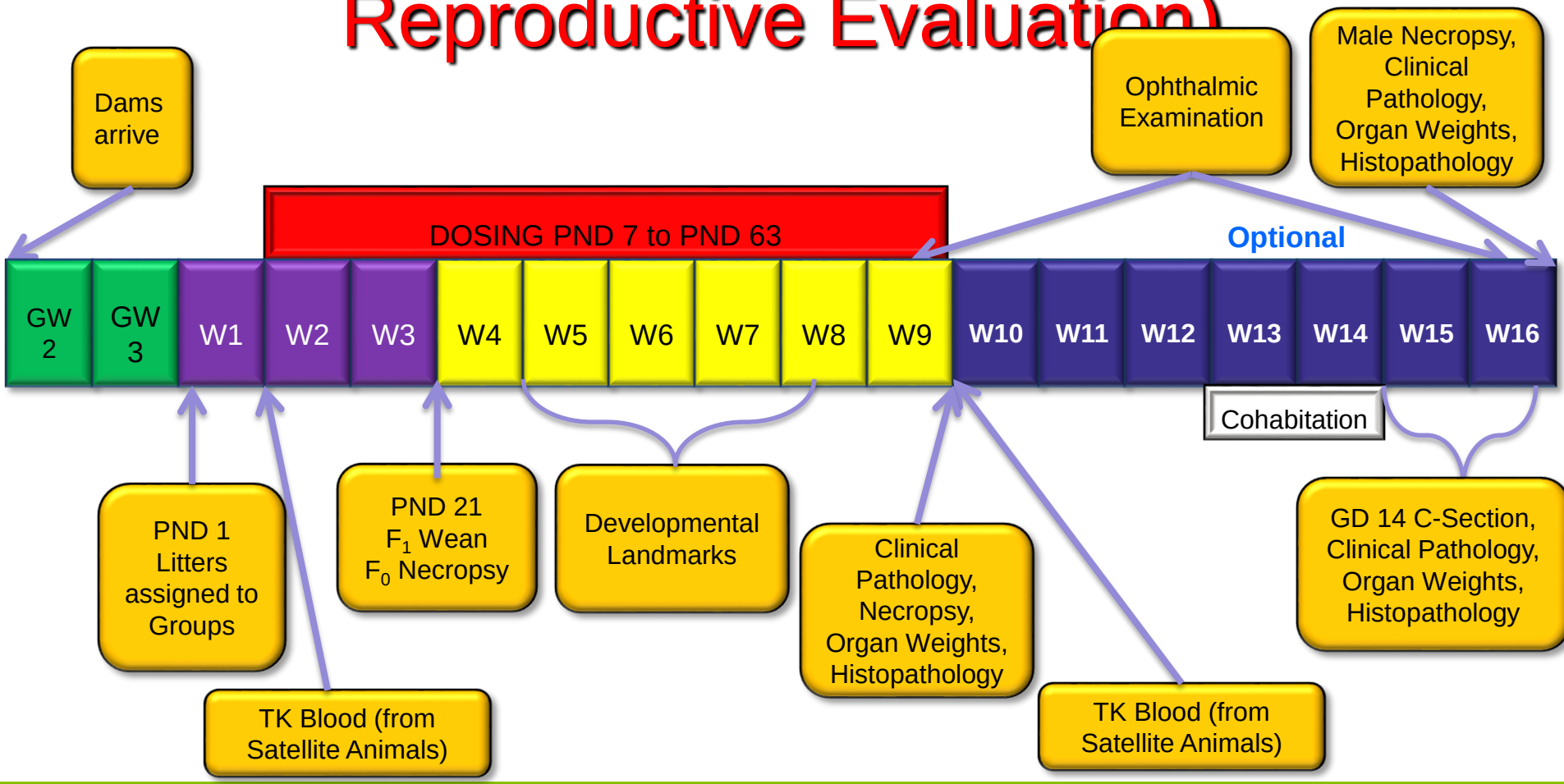
Juvenile Toxicity Study Design (Targeted Liver)



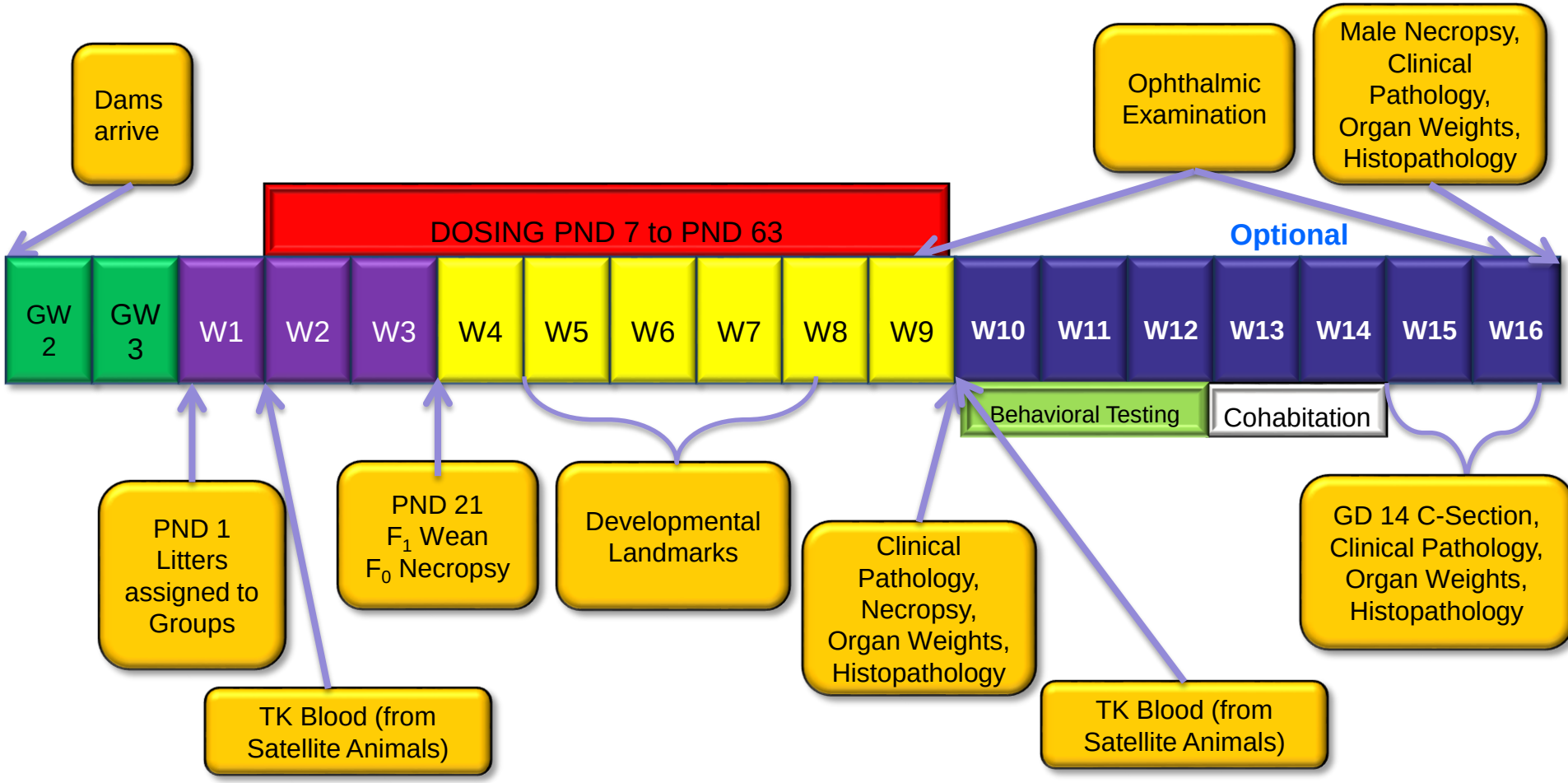
Juvenile Toxicity Study Design (General Toxicity)



Juvenile Toxicity Study Design (General Toxicity with Reproductive Evaluation)

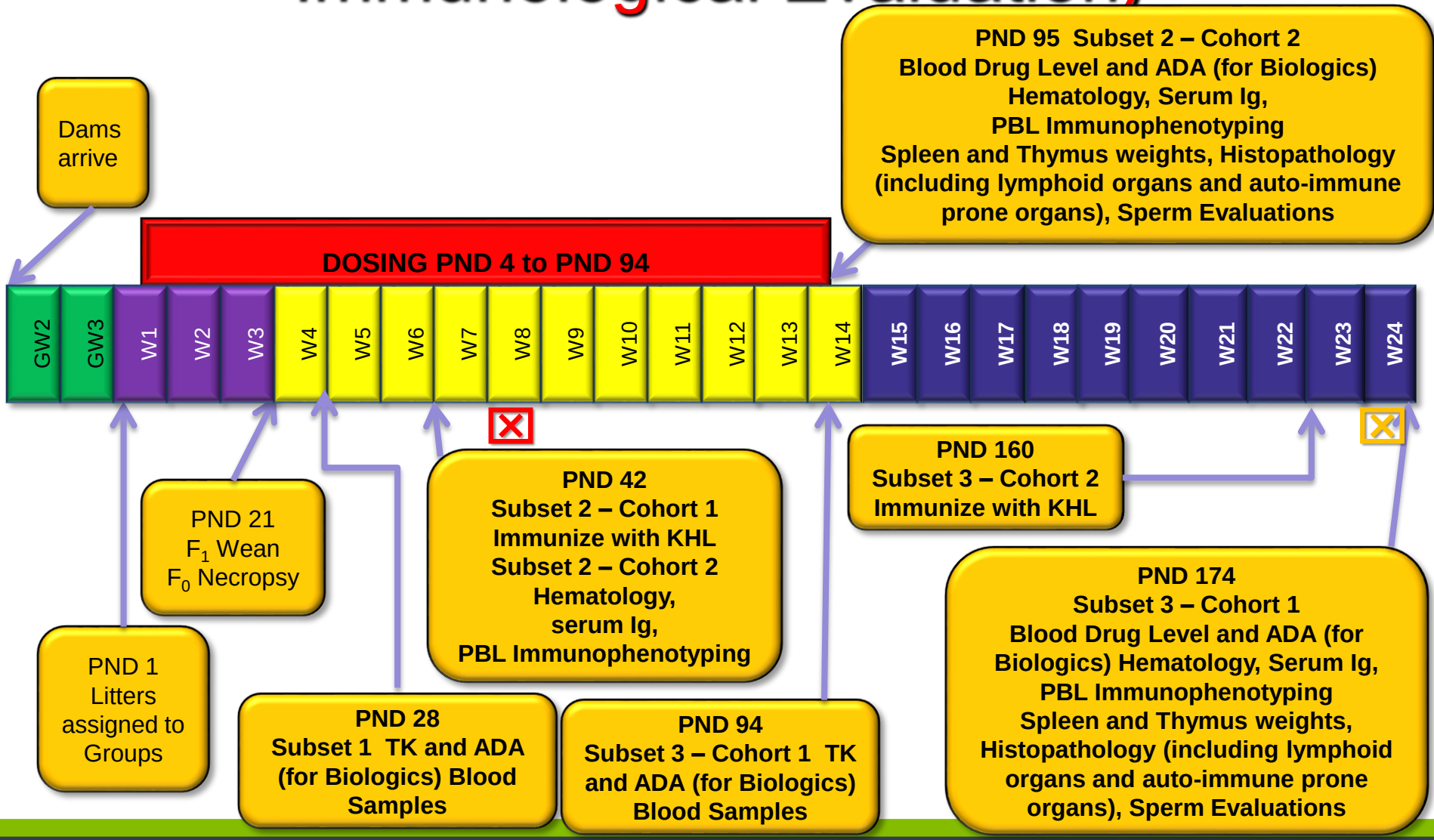


Juvenile Toxicity Study Design (Targeted CNS and Reproductive Evaluation)



May require satellite groups for Brain Perfusion and Neurohistopathology

Juvenile Toxicity Study Design (Targeted Immunological Evaluation)



Body Weight Intervals and Dosing

PND	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
Males	16.8	19.1	21.5	23.8	26.2	28.9	31.6	34	36.6	39	41.5	43.9	46.7	50.7	54.9	57.8	
Females	15.8	18	20.2	22.6	25	27.6	30.1	32.5	34.9	37.2	39.4	41.8	44.6	48.2	52.6	55.1	
Weight every 2nd day (males)	100.0%	88.0%	100.0%	90.3%	100.0%	90.7%	100.0%	92.9%	100.0%	93.8%	100.0%	94.5%	100.0%	92.1%	100.0%	95.0%	
Weight every 2nd day (females)	87.8%	87.8%	100.0%	89.4%	100.0%	90.6%	100.0%	92.6%	100.0%	93.8%	100.0%	94.3%	100.0%	92.5%	100.0%	95.5%	
Weight every 3rd day (males)	100.0%	88.0%	78.1%	100.0%	90.8%	82.4%	100.0%	92.9%	86.3%	100.0%	94.0%	88.8%	100.0%	92.1%	85.1%	100.0%	
Weight every 3rd day (males)	100.0%	87.8%	78.2%	100.0%	90.4%	81.9%	100.0%	92.6%	86.2%	100.0%	94.4%	89.0%	100.0%	92.5%	84.8%	100.0%	
weight twice per week (males)	100.0%	88.0%	78.1%	70.6%	100.0%	90.7%	82.9%	100.0%	92.9%	87.2%	81.9%	100.0%	94.0%	86.6%	100.0%	95.0%	
weight twice per week (females)	100.0%	87.8%	78.2%	69.9%	100.0%	90.6%	83.1%	100.0%	93.1%	87.4%	82.5%	100.0%	93.7%	86.7%	100.0%	95.5%	
Weight weekly (males)	100.0%	88.0%	78.1%	70.6%	64.1%	58.1%	53.2%	100.0%	92.9%	87.2%	81.9%	77.4%	72.8%	67.1%	100.0%	95.0%	
Weight weekly (males)	100.0%	87.8%	78.2%	69.9%	63.2%	57.2%	52.5%	100.0%	93.1%	87.4%	82.5%	77.8%	72.9%	67.4%	100.0%	95.5%	

PND	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	
Males						2.6	99.8	107.5	115.7	124	132.6	140.9	150.8	159.1	167.8	177.4	
Females						6.2	92.1	98.2	104.7	111.1	117.9	124.5	131	136.5	142.4	148.2	
Weight every 2n						.5%	100.0%	92.8%	100.0%	93.3%	100.0%	94.1%	100.0%	94.8%	100.0%	94.6%	
Weight every 2n						.5%	100.0%	93.8%	100.0%	94.2%	100.0%	94.7%	100.0%	96.0%	100.0%	96.1%	
Weight every 3r						0.0%	92.8%	86.1%	100.0%	93.3%	87.3%	100.0%	93.4%	88.6%	100.0%	94.6%	
Weight every 3r						0.0%	93.6%	87.8%	100.0%	94.2%	88.8%	100.0%	95.0%	91.2%	100.0%	96.1%	
weight twice pe						0.0%	92.8%	86.1%	80.0%	100.0%	93.5%	88.0%	100.0%	94.8%	89.9%	85.0%	
weight twice pe						0.0%	93.6%	87.8%	82.3%	100.0%	94.2%	89.2%	100.0%	96.0%	92.0%	88.4%	
Weight weekly (						0.0%	92.8%	86.1%	80.0%	74.7%	69.8%	65.7%	100.0%	94.8%	89.9%	85.0%	
Weight weekly (						0.0%	93.6%	87.8%	82.3%	77.6%	73.1%	69.2%	100.0%	96.0%	92.0%	88.4%	

	44	45	46	47	48	49	50	51	52	53	54	
Males	29.3	238.2	246.5	255.3	263.7	273.5	281.5	290.4	300.4	307.2	316.7	
Females	77.4	181	185.2	188.6	192.7	196.5	200.7	204.6	208.2	211.3	214.5	
Weight every 2n	.2%	100.0%	96.6%	100.0%	96.8%	100.0%	97.2%	100.0%	96.7%	100.0%	97.0%	
Weight every 2n	.4%	100.0%	97.7%	100.0%	97.9%	100.0%	97.9%	100.0%	98.3%	100.0%	98.5%	
Weight every 3r	.2%	92.6%	100.0%	96.6%	93.5%	100.0%	97.2%	94.2%	100.0%	97.8%	94.9%	
Weight every 3r	.4%	95.4%	100.0%	98.2%	96.1%	100.0%	97.9%	96.0%	100.0%	98.5%	97.1%	
weight twice pe	.8%	89.4%	100.0%	96.6%	93.5%	100.0%	97.2%	94.2%	91.0%	100.0%	97.0%	
weight twice pe	.%	.0%	100.0%	98.2%	96.1%	100.0%	97.9%	96.0%	94.4%	100.0%	98.5%	
Weight weekly (	.8%	.4%	.4%	.4%	80.7%	100.0%	97.2%	94.2%	91.0%	89.0%	86.4%	
Weight weekly (	.3%	93.4%	.8%	.8%	.8%	100.0%	97.9%	96.0%	94.4%	93.0%	91.6%	

	60	61	62	63	64	68	69	70	% of correct dose
Males	60.2	367.3	372.3	379.5	385.7	393	399.5	424.6	
Females	234	236.2	238.7	242.3	245.1	248.3	250	253.7	
Weight every 2n	.2%	100.0%	98.7%	100.0%	98.4%	100.0%	98.4%	100.0%	98.6%	97.5%
Weight every 2n	.8%	100.0%	99.0%	100.0%	98.9%	100.0%	99.3%	100.0%	98.9%	97.9%
Weight every 3rd day (males)	100.0%	98.4%	95.6%	100.0%	97.7%	95.9%	100.0%	98.7%	96.8%	100.0%	98.1%	96.5%	100.0%	98.6%	97.1%	100.0%	95.1%
Weight every 3rd day (males)	100.0%	98.5%	97.2%	100.0%	98.4%	97.2%	100.0%	99.0%	97.5%	100.0%	98.7%	98.0%	100.0%	98.9%	98.1%	100.0%	95.7%
weight twice per week (males)	94.8%	100.0%	97.1%	95.3%	93.1%	100.0%	98.1%	96.7%	100.0%	98.4%	96.6%	95.0%	100.0%	98.6%	97.1%	100.0%	93.8%
weight twice per week (females)	96.7%	100.0%	98.7%	97.6%	96.0%	100.0%	99.1%	98.0%	100.0%	98.9%	97.6%	96.9%	100.0%	98.9%	98.1%	100.0%	94.6%
Weight weekly (males)	84.4%	100.0%	97.1%	95.3%	93.1%	91.4%	89.7%	88.5%	100.0%	98.4%	96.6%	95.0%	93.5%	92.1%	90.8%	100.0%	86.4%
Weight weekly (males)	89.9%	100.0%	98.7%	97.6%	96.0%	94.9%	94.0%	93.0%	100.0%	98.9%	97.6%	96.9%	95.5%	94.5%	93.7%	100.0%	88.2%

PND	% of correct dose
Males	
Females	
Weight every 2nd day (males)	97.5%
Weight every 2nd day (females)	97.9%
Weight every 3rd day (males)	95.1%
Weight every 3rd day (males)	95.7%
weight twice per week (males)	93.8%
weight twice per week (females)	94.6%
Weight weekly (males)	86.4%
Weight weekly (males)	88.2%

Female Postnatal Body Weight and Calculated Blood Volume

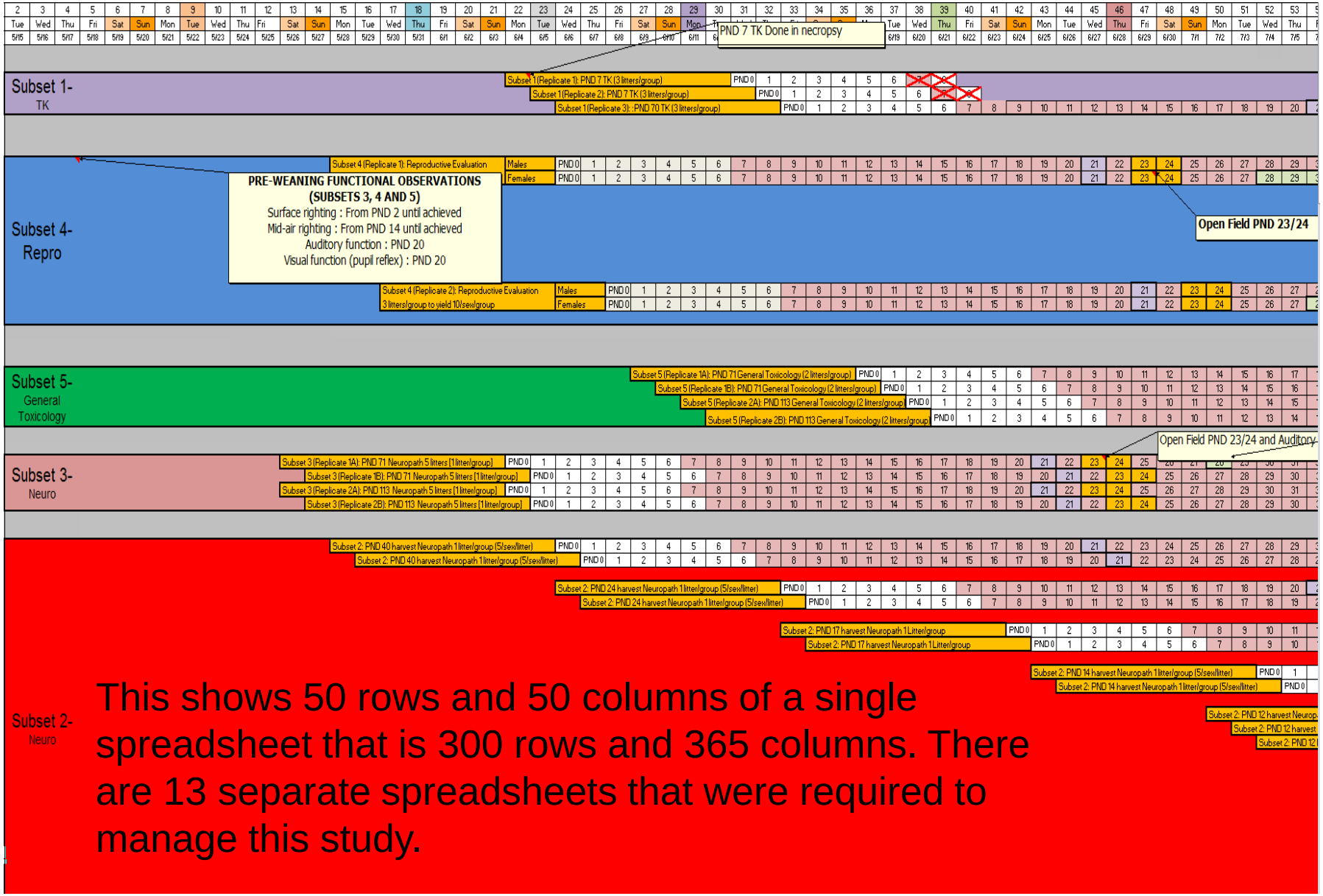
	PND 7	PND 8	PND 9	PND 10	PND 11	PND 12	PND 13	PND 14	PND 15	PND 16	PND 17	PND 18	PND 19	PND 20	PND 21
Female Mean Body Weight (gms)	15.8	18.0	20.2	22.6	25.0	27.6	30.1	32.5	34.9	37.2	39.4	41.8	44.6	48.2	52.6
Female TBV (mL)	1.01	1.15	1.29	1.45	1.60	1.77	1.93	2.08	2.23	2.38	2.52	2.68	2.85	3.08	3.37
Female 7.5% TBV (mL)	0.08	0.09	0.10	0.11	0.12	0.13	0.14	0.16	0.17	0.18	0.19	0.20	0.21	0.23	0.25
Female 10% TBV (mL)	0.10	0.12	0.13	0.14	0.16	0.18	0.19	0.21	0.22	0.24	0.25	0.27	0.29	0.31	0.34
Female 15% TBV (mL)	0.15	0.17	0.19	0.22	0.24	0.26	0.29	0.31	0.34	0.36	0.38	0.40	0.43	0.46	0.50
Female 20% TBV (mL)	0.20	0.23	0.26	0.29	0.32	0.35	0.39	0.42	0.45	0.48	0.50	0.54	0.57	0.62	0.67

	PND 22	PND 23	PND 24	PND 25	PND 26	PND 27	PND 28	PND 29	PND 30	PND 31	PND 32	PND 33	PND 34	PND 35	PND 36
Female Mean Body Weight (gms)	55.1	59.7	64.2	69.5	74.9	80.6	86.2	92.1	98.2	104.7	111.1	117.9	124.5	131.0	136.5
Female TBV (mL)	3.53	3.82	4.11	4.45	4.79	5.16	5.52	5.89	6.28	6.70	7.11	7.55	7.97	8.38	8.74
Female 7.5% TBV (mL)	0.26	0.29	0.31	0.33	0.36	0.39	0.41	0.44	0.47	0.50	0.53	0.57	0.60	0.63	0.66
Female 10% TBV (mL)	0.35	0.38	0.41	0.44	0.48	0.52	0.55	0.59	0.63	0.67	0.71	0.75	0.80	0.84	0.87
Female 15% TBV (mL)	0.53	0.57	0.62	0.67	0.72	0.77	0.83	0.88	0.94	1.01	1.07	1.13	1.20	1.26	1.31
Female 20% TBV (mL)	0.71	0.76	0.82	0.89	0.96	1.03	1.10	1.18	1.26	1.34	1.42	1.51	1.59	1.68	1.75

	PND 37	PND 38	PND 39	PND 40	PND 41	PND 42	PND 43	PND 44	PND 45	PND 46	PND 47	PND 48	PND 49	PND 50	PND 51
Female Mean Body Weight (gms)	142.4	148.2	153.8	159.5	164.4	169.1	172.7	177.4	181.0	185.2	188.6	192.7	196.5	200.7	204.6
Female TBV (mL)	9.11	9.48	9.84	10.21	10.52	10.82	11.05	11.35	11.58	11.85	12.07	12.33	12.58	12.84	13.09
Female 7.5% TBV (mL)	0.68	0.71	0.74	0.77	0.79	0.81	0.83	0.85	0.87	0.89	0.91	0.92	0.94	0.96	0.98
Female 10% TBV (mL)	0.91	0.95	0.98	1.02	1.05	1.08	1.11	1.14	1.16	1.19	1.21	1.23	1.26	1.28	1.31
Female 15% TBV (mL)	1.37	1.42	1.48	1.53	1.58	1.62	1.66	1.70	1.74	1.78	1.81	1.85	1.89	1.93	1.96
Female 20% TBV (mL)	1.82	1.90	1.97	2.04	2.10	2.16	2.21	2.27	2.32	2.37	2.41	2.47	2.52	2.57	2.62

	PND 52	PND 53	PND 54	PND 55	PND 56	PND 57	PND 58	PND 59	PND 60	PND 61	PND 62	PND 63	PND 64	PND 65	PND 66
Female Mean Body Weight (grams)	208.2	211.3	214.5	218.6	222.0	224.9	227.5	231.2	234.0	236.2	238.7	242.3	245.1	248.3	250.0
Female TBV (mL)	13.32	13.52	13.73	13.99	14.21	14.39	14.56	14.80	14.98	15.12	15.28	15.51	15.69	15.89	16.00
Female 7.5% TBV (mL)	1.00	1.01	1.03	1.05	1.07	1.08	1.09	1.11	1.12	1.13	1.15	1.16	1.18	1.19	1.20
Female 10% TBV (mL)	1.33	1.35	1.37	1.40	1.42	1.44	1.46	1.48	1.50	1.51	1.53	1.55	1.57	1.59	1.60
Female 15% TBV (mL)	2.00	2.03	2.06	2.10	2.13	2.16	2.18	2.22	2.25	2.27	2.29	2.33	2.35	2.38	2.40
Female 20% TBV (mL)	2.66	2.70	2.75	2.80	2.84	2.88	2.91	2.96	3.00	3.02	3.06	3.10	3.14	3.18	3.20

	PND 67	PND 68	PND 69	PND 70	PND 73	PND 77	PND 80	PND 84	PND 87	PND 91	PND 94	PND 98	PND 101	PND 105	PND 108
Female Mean Body Weight (gms)	253.7	256.4	258.5	260.9	265.6	274.1	281.0	287.3	291.3	297.7	303.7	307.0	310.8	316.9	319.7
Female TBV (mL)	16.24	16.41	16.54	16.70	17.00	17.54	17.98	18.39	18.64	19.05	19.44	19.65	19.89	20.28	20.46
Female 7.5% TBV (mL)	1.22	1.23	1.24	1.25	1.27	1.32	1.35	1.38	1.40	1.43	1.46	1.47	1.49	1.52	1.53
Female 10% TBV (mL)	1.62	1.64	1.65	1.67	1.70	1.75	1.80	1.84	1.86	1.91	1.94	1.96	1.99	2.03	2.05
Female 15% TBV (mL)	2.44	2.46	2.48	2.50	2.55	2.63	2.70	2.76	2.80	2.86	2.92	2.95	2.98	3.04	3.07
Female 20% TBV (mL)	3.25	3.28	3.31	3.34	3.40	3.51	3.60	3.68	3.73	3.81	3.89	3.93	3.98	4.06	4.09



This shows 50 rows and 50 columns of a single spreadsheet that is 300 rows and 365 columns. There are 13 separate spreadsheets that were required to manage this study.

Example: Comparative Development of the Kidney

Timing of Nephrogenesis Completion for Various Species

Species	Nephrogenesis Completion
Man	35 weeks gestation
Sheep	Before birth
Guinea Pig	Before birth
Dog	Postnatal week 2
Pig	Postnatal week 3
Mouse	Before birth
Rat	Postnatal week 4-6

Critical period not covered with standard studies

Data from T. Zoetis, M.E. Hurtt. Species comparison of anatomical and functional renal development Birth Defects Research (Part B) 68:111-120 (2003)

Ontogeny and Pharmacology

- Excretory organ (liver and kidneys) development has the greatest impact on drug disposition (pharmacokinetics)
- The most dramatic changes occur during the first days to months of life
- Anticipate age-related differences in drug disposition based on knowledge of ontogeny
- Effect of ontogeny on tissue/organ sensitivity to drugs (pharmacodynamics) is poorly studied
- Disease states may alter a drug's PK/PD

Trends in TK Function by Age Groups Compared with Adults

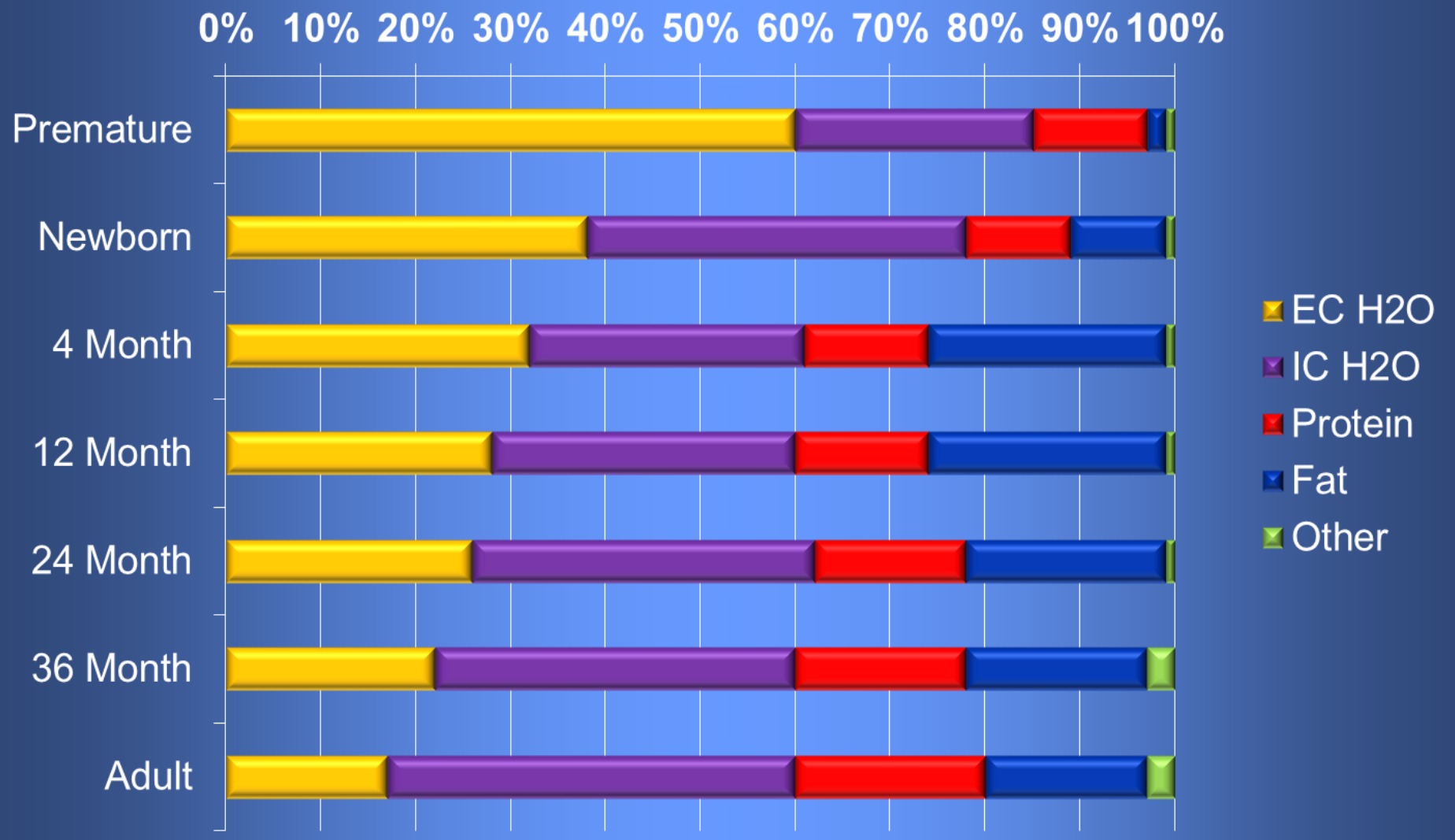
Clearance Pathway Function	Premature Neonates	Neonatal <1 month	Early Infant 1-5 months	Late Infant 6-11 months	Toddler 1-2 years	Older Children
Oral Absorption	↑	↑	↑	↔	↔	↔
Dermal Absorption	↑	↔	↔	↔	↔	↔
Lung Absorption	↑	↑	↑	↑	↑	↑
Renal Clearance	NA	↓	↓/↔	↔	↔	↔
CYP1A2	↓	↓	↓/↔	↑**Scale:BW$^{3/4}$**	↑**Scale:BW$^{3/4}$**	↑**Scale:BW$^{3/4}$**
CYP2E1	↓	↓	↓/↔	↑**Scale:BW$^{3/4}$**	↑**Scale:BW$^{3/4}$**	↑**Scale:BW$^{3/4}$**
CYP3A family (except 3A7)	↓	↓	↓/↔	↑**Scale:BW$^{3/4}$**	↑**Scale:BW$^{3/4}$**	↑**Scale:BW$^{3/4}$**
CYP3A7	↑	↑	↔	↓=	↓=	↓=
Other CYPs	↓	↓	↓/↔	↑**Scale:BW$^{3/4}$**	↑**Scale:BW$^{3/4}$**	↑**Scale:BW$^{3/4}$**
Glucuronidation	↓	↓	↓/↔	↔	↔	↔
N-Acetylation	↓	↓	↓	↓	↔	↔
Glutathione transferases	**Uncertain**	**Uncertain**	**Uncertain**	↔	↔	↔
Epoxide hydrolase	↓	↓	↓/↔	↔	↔	↔
Alcohol Dehydrogenase	↓	↓	↓/↔	↑**Scale:BW$^{3/4}$**	↑**Scale:BW$^{3/4}$**	↑**Scale:BW$^{3/4}$**

Adapted from Ginsberg et al, 2004

Absorption (Human)

- Physicochemical properties of the drug - lipid vs. water solubility
- ↑ V_d (Volume of Distribution)
  - Small size, large Surface Area: Body Wt
  - Body composition (Extracellular H_2O, Intercellular H_2O, fat, protein)
- ↑ Gastric pH (↑basic / ↓acid drug absorption)
- ↓ Gastric emptying

Ontogeny of Body Composition



Adapted from Kaufman, Pediatric Pharmacology (Yaffe & Aranda, eds) pp. 212-9, 1992

Absorption (Human)

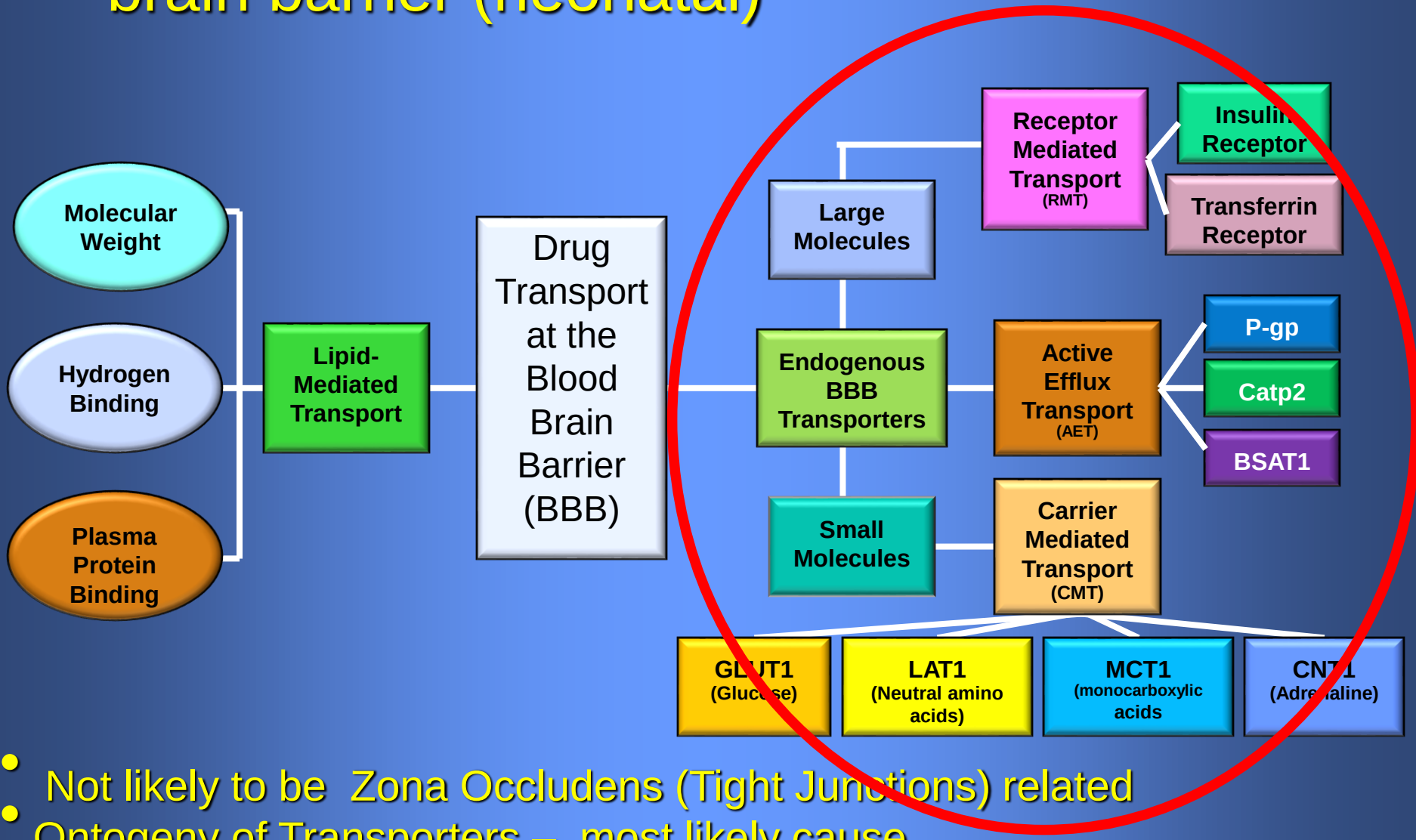
- Variable GI motility
  - Infant/neonate (Low)
  - Child (High)
- Bioavailability unpredictable
  - Topical effect is increased in neonates
  - IM absorption is faster
  - Inhalation absorption is increased
  - Gastrointestinal
    - Passive diffusion
    - Transporters

Distribution (Human)

- Tissue binding differences with age (receptor, protein ↓)
  - Determines the concentration of free drug
- Unique exposure routes
  - Placenta
  - Milk
- Ontogeny of Transporters
- Intracellular depots

Distribution (Human)

- More drugs enter the immature blood brain barrier (neonatal)



- Not likely to be Zona Occludens (Tight Junctions) related
- Ontogeny of Transporters – most likely cause

Adapted from numerous sources

Metabolism (Human)

- Immature Liver
  - First pass effect is decreased
- Phase I Maturation (0-3 Years)
  - Enzyme activity varies with P450, substrate, age
- Phase II Maturation (0-12 Years)
- Ontogeny of Transporters
- Active metabolites

Excretion (Human)

- Generally depend on Transporters
- Immature Kidneys (full kidney function at 2-3 years)
  - ↓ GFR
  - ↓ tubular secretion/reabsorption
  - Perfusion to the kidneys may be ↓
  - Infant/Neonate: slower CL, longer T1/2
  - Child: rapid CL, shorter T1/2
- Biliary

Internal Exposure Determinations in Developmental and Juvenile Studies

- Internal exposures more relevant for assessing toxicity than the external dose
- May be required to have several TK intervals (and different dose levels) to correspond with different ages/maturity of ADME components
- Toxicokinetic parameters very useful for age and cross-species comparisons
- Characterizing plasma levels of parent drug and relevant metabolites may clarify whether or not selective functional/developmental changes do occur, or whether changes actually occur at same or even at greater internal exposures than in adults.

Pathology Evaluation: Major Challenges in Juvenile Studies

Postnatal tissue development during study

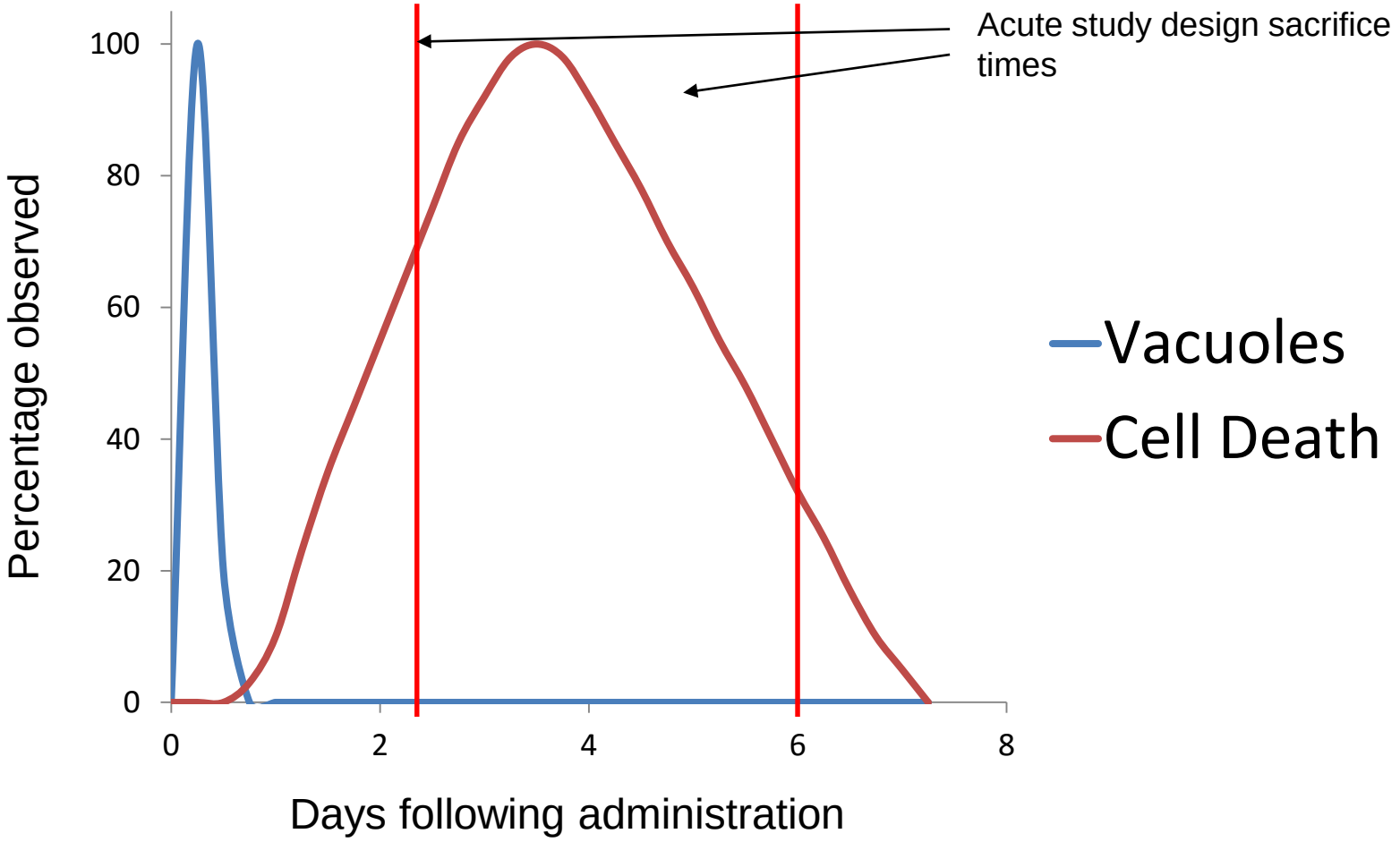
- Absence of concurrent controls for unscheduled deaths
- Causes of histologic alteration in unscheduled deaths:
  - Direct effect of test article
  - Indirect effect of test article [inappetance, stress (immune system)]
  - Delayed development (e.g. endocrine disruption)
  - Normal postnatal histogenesis
- Presence of intercurrent disease

Neurohistopathology of Postnatal IP Dose of MK-801 in Juvenile Rats

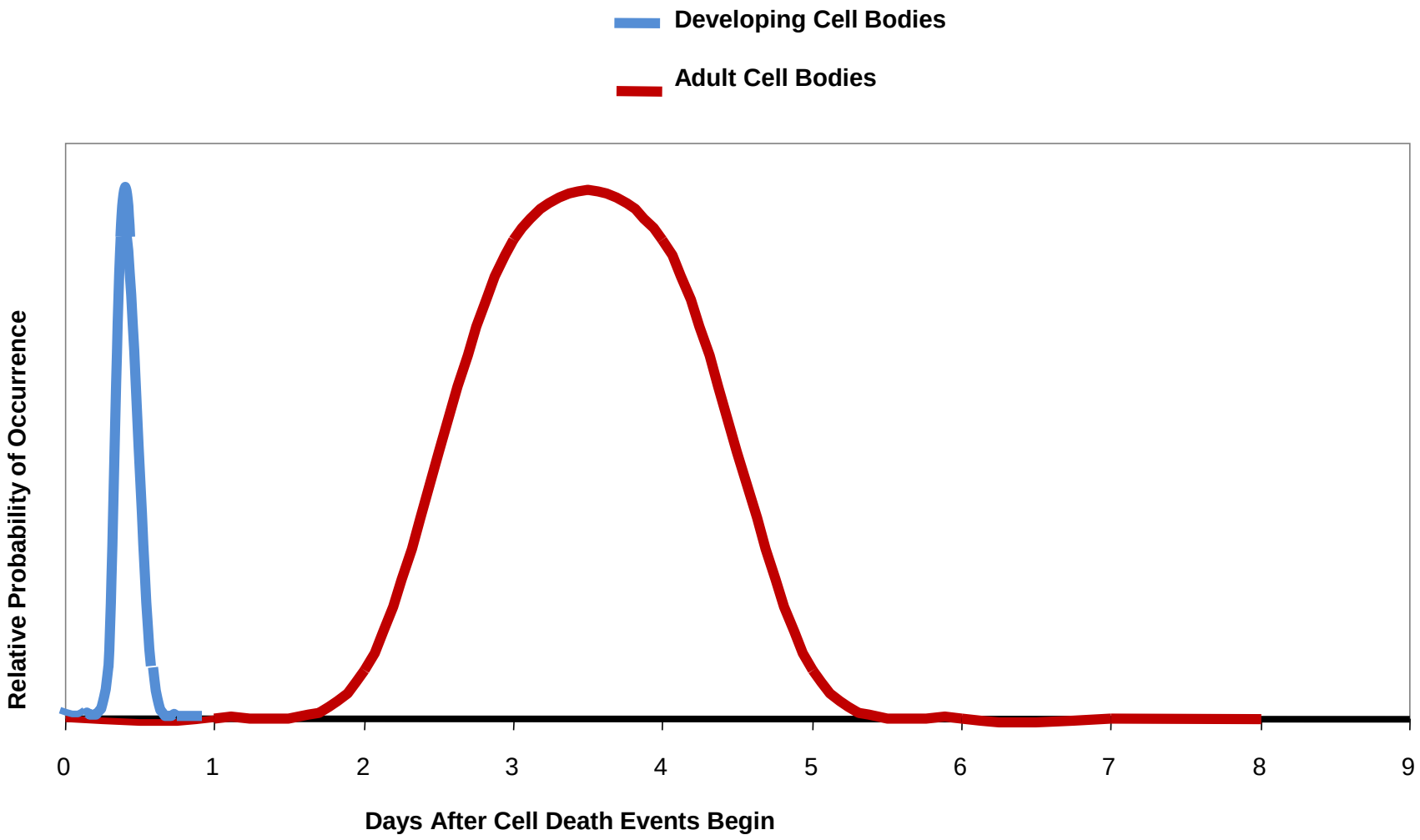
Introduction

- Characterization of low occurrence of isolated minimal or mild degeneration and apoptosis in control brains is imperative to determine the difference between relationship of normal changes and the effects of a test article.
- MK-801 (dizocilpine maleate) is a potent non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist known to induce neurotoxicity (neuronal degeneration and apoptosis) in the rat while it has also been shown to be neuroprotective in several animal models of pathologies that involve hyperactivation of the MDMA receptors, such as stroke, ischemia, epilepsy, and neuropathic pain (Bender, 2010).
- Olney et al (1989) observed transient intracytoplasmic vacuoles in rat brains following MK-801 administration. Olney et al. (1990, 1993) and Fix et al. (1994) further observed that MK-801 caused neuronal degeneration that was co-located at vacuole sites particularly in the retrosplenal corftex.
- Importantly, neurodegeneration was also found in regions of the brain distant from the vacuole sites. In a time-course study using adult Wistar rats Horvath et al. (1997) determined that lesions could be observed within hours of the dosage administration but were no longer observable after 4 days.
- For the early postnatal rats, the period of observable lesion is diminished.
- Jevtovic-Todorovic, V., et al. (2000, 2003a, 2003b) demonstrated that exposure of the developing brain to anesthetic agents that block NMDA receptors during the period of synaptogenesis can trigger widespread apoptotic neurodegeneration.
- During development in the rat, especially during postnatal days (PND) 7–14, the central nervous system exhibits enhanced susceptibility to the toxic effects of modulation of the NMDA receptor system. This enhanced susceptibility has been suggested to be derived from the increased expression of specific NMDA receptor subunits (Miyamoto et al., 2001).
- Neonatal rats administered the MK-801 during the first two weeks of life have been shown to develop abnormal axonal arborization in the retinal connections to the superior colliculus, interfering with normal visual responses (Haberny, 2002).

The peak observable time of degeneration following administration of MK-801 to adult rat lasts ~ 3 days.



In the developing brain the window of opportunity for measurable neurodegeneration is shrunk from days to hours



Key Points:

- Degeneration and apoptosis in early neonatal rats changes rapidly (within a day); evaluation of changes resulting from administration of a test chemical must account for degeneration and apoptosis as seen in age matched controls.
- Apoptosis that was present in MK-801 treated rats was generally distinct and unequivocal when compared with the control rats.
- Degeneration (as shown by the CuAg degeneration stain) was generally more sensitive in detecting changes as compared to apoptosis (as shown in the caspase 9 stain) in both numbers of involved sites and intensity of changes within involved sites.
- Based on this data, isolated minimal or mild occurrences of degeneration and apoptosis should not be considered treatment related events in juvenile Sprague-Dawley rats.
- Degeneration and apoptosis were present in numerous brain sites in MK-801 treated rats in the earlier PNDs (PNDs 8 through 14), intermediate at PND 17, and few sites were observed at PNDs 24, 40, 71 and 113 and females generally had more brain sites involved than did males.

Methods

- The MK-801 group received a single dose (3 mg/kg, IP) on PNDs 7, 8, 9, 11 13, 16, 23, 39, 69 or 111). The control article (distilled water, 5 mL/Kg, oral gavage) was dosed daily from PND 7 until the day prior to termination. Thirty or twenty rats per sex per time-point had brains perfused/harvested on PNDs 8, 9, 10 and 12, or on PNDs 14, 17, 24, 40, 71 and 113, respectively. The brains were embedded, coronally sectioned at 40µ (through the entire brain length), and stained with amino cupric silver (degeneration changes) and caspase 9 stain (apoptosis). The disintegrative degeneration stain was chosen for this study because it has been demonstrated to be effective in assessing for the neurodegeneration associated with Olney lesions. Brains (PNDs 8 to 40 only) were evaluated for apoptosis using a caspase-9 antibody stain because increased apoptosis is believed to be the main adverse effect for this drug class (N-methyl-D-aspartate [NMDA] receptor antagonist neuropathology) at this age in rats of both genders. Neurohistopathological evaluation of the entire brain for degeneration and apoptosis was performed.

Perfusion

- Rats were perfusion-fixed following euthanasia. Animals were transcardially perfused with a perfusion wash, followed by a perfusion fixative.
- The heads were removed and stored in the fixative for 18-24 hours after which the brains were removed and stored in buffer.
- Appropriate sized conical vials were used for storage once the brains had been removed from the cranium.
- Three different perfusion fixative were use based on age of the pup.

Histology

- The brains were shipped in Na Cacodylate Buffer Solution, at room temperature for processing.
- Specimens were examined, trimmed to yield the cerebrum, then treated overnight with 20% glycerol and 2% dimethylsulfoxide to prevent freeze-artifacts.
- The specimens were then multiply embedded, with up to 16 brains per block in a gelatin matrix using MultiBrain® Technology.
- The blocks were rapidly frozen, after curing by immersion in isopentane chilled with crushed dry ice and mounted on a freezing stage of an AO 860 sliding microtome.
- The MultiBrain® blocks were sectioned coronally at 40µ.
- All sections were cut through the entire length of the trimmed brains and collected sequentially into a 4x6 array of containers filled with Antigen Preserve; no sections were discarded.

Caspase-9 Antibody Stain

- Brains (PNDs 8 to 40 only) were evaluated for apoptosis using a caspase-9 antibody stain because increased apoptosis is believed to be the main adverse effect for this drug class (N-methyl-D-aspartate [NMDA] receptor antagonist neuropathology) at this age in rats of both genders.
- Every 8th section was stained free-floating for immunochemistry. All incubation solutions from the blocking serum onward use Tris buffered saline (TBS) with Triton X100 as the vehicle; all rinses are with TBS.
- After a hydrogen peroxide treatment and blocking serum, the sections were immunostained with the primary antibodies, as shown in the table below, overnight at room temperature.
- Vehicle solutions contained TritonX-100 for permeabilization. Following rinses, a biotinylated secondary antibody (anti IgG of host animal in which the primary antibody was produced) was applied. After further rinses, Vecta's ABC solution (avidin-biotin-HRP complex) was applied.
- The sections were again rinsed, then treated with diaminobenzidine tetrahydrochloride (DAB) and hydrogen peroxide to create a visible reaction product.
- Following further rinses, the sections were mounted on gelatin coated glass slides, air dried, dehydrated in alcohols, cleared in xylene and coverslipped.

Histopathologic Evaluation of the Brain

- Degeneration and apoptosis were graded using a 4 point scale i.e. 1 (minimal), 2 (mild), 3 (moderate), and 4 (marked).
- Location of degeneration and apoptosis (in excess of vehicle control levels) within the brain was recorded.
- The following areas were evaluated: subiculum, cingulate cortex, retrosplenial cortex, entorhinal cortex, olfactory nucleus, frontal cortex, insular cortex, perirhinal cortex, piriform cortex, the temporal cortex, orbital cortex, primary and secondary motor cortex, claustrum, amygdala, thalamus, ventral auditory cortex, entorhinal cortex, substantia nigra and mammillary body.

Results:

- The current data describes the neurodegeneration and apoptosis in the juvenile rat brain caused by acute MK-801 treatment at different intervals (see slides below).
- Degeneration and apoptosis in MK-801 treated rats were present in a large number of brain sites in the earlier PNDs especially PNDs 8, 9, 10, 12 and 14 with severities ranging from minimal to marked.
- These changes at later PNDs were substantially diminished. Females generally had more numerous brain sites involved than did males especially at the later PNDs.
- By PND 40, males typically had only 3 or 4 sites at which degeneration was observed. Apoptosis was present at minimal severity in only 2 sites at PND 40 in MK-801 treated males. Otherwise, degeneration and apoptosis were present in positive control males and females at all PNDs.
- Some control animals exhibited greater degeneration and to a greater extent, apoptosis, in numerous brain sites that were greater than levels within the remaining animals of that sex/age group. This occurred at the earlier PNDs, most prominently from PND 8 through PND 24. The location and severity of the lesions are presented. Based on this data, isolated minimal or mild occurrences of degeneration and apoptosis should not be considered treatment related events in juvenile Sprague-Dawley rats.

Sites at Which Degeneration Was Observed in MK-801 Male Rats using the AmCuAg Degeneration stain

Age (Postnatal days)	**8**	**9**	**10**	**12**	**14**	**17**	**24**	**40**	**71**	**113**
No.Animals with Lesions	**16**	**16**	**16**	**16**	**16**	**16**	**16**	**13**	**13**	**21**
Number Examined	**16**	**16**	**16**	**16**	**16**	**16**	**17**	**16**	**16**	**21**
Entorhinal cortex	12	12	15	9	8	0	5	0	0	1
Cingulate cortex	16	16	13	16	11	0	0	0	7	15
Lateral cortex anterior	16	16	16	13	0	0	0	0	0	0
Prelimbic cortex	8	13	4	11	1	0	0	0	0	0
Infralimbic/prelimbic cortex	0	0	0	1	1	0	0	0	0	0
Piriform cortex	3	2	0	9	9	6	0	7	1	5
Globus pallidus	16	11	12	11	2	0	0	0	0	0
Thalamic nuclei	16	16	16	14	12	0	0	0	0	0
Presubiculum	9	14	15	9	2	0	0	0	0	0
Postsubiculum	13	16	16	15	1	0	0	0	0	0
Hippocampus	16	15	15	16	9	0	0	0	0	0
Somatosensory cortex	0	0	0	1	2	0	0	0	0	0
Retrosplenial cortex	16	16	16	16	16	5	0	6	13	21
Mammillary nuclei	14	11	14	15	15	7	0	0	0	0
Lateral cortex middle/posterior	15	16	16	16	15	0	0	0	0	0
Frontal association cortex	14	13	11	12	1	0	0	0	0	0
Septal nuclei	7	4	6	5	5	0	0	0	0	0
Accumbens	16	16	16	13	1	0	0	0	0	0
Pontine nuclei	15	16	10	11	12	2	0	0	0	0
Motor cortex	16	15	15	16	1	0	0	0	0	0
Caudate putamen	16	16	15	12	0	0	0	0	0	0
Dentate	1	4	0	4	3	7	0	2	0	0
Hypothalamic nuclei	15	12	7	9	4	0	0	0	0	0
Amygdaloid nuclei	16	15	14	10	0	0	0	0	0	0
Dorsal peduncular nucleus	6	9	2	9	0	0	0	0	0	0
Dorsal peduncular cortex	0	0	2	0	0	0	0	0	0	0
Bed nuclei stria terminalis	2	1	0	2	2	0	0	0	0	0
Dorsal tenia tecta	9	7	5	3	0	0	0	0	0	0
Orbital cortex	1	5	5	1	0	0	0	0	0	0
Ventral pallidum	1	0	1	0	1	0	0	0	0	0
Claustrum	1	1	0	0	0	0	0	0	0	0
Navicular nuclei	0	1	1	0	0	0	0	0	0	0

Sites at Which Degeneration Was Observed in MK-801 Female Rats using the AmCuAg Degeneration stain

Age (Postnatal days)	8	9	10	12	14	17	24	40	71	113
No.Animals with Lesions	16	16	16	16	16	16	18	16	16	21
Number Examined	16	16	16	16	16	16	18	16	16	21
Olfactory lobe	6	8	6	15	7	5	8	8	13	8
Olfactory bulb	0	0	0	0	8	5	9	8	0	0
Olfactory nucleus	0	1	0	0	0	0	1	0	0	0
Olfactory tubercle	0	0	0	2	0	0	0	0	0	0
Entorhinal cortex	9	11	13	14	13	3	5	9	15	20
Cingulate cortex	15	14	14	16	14	0	0	1	10	19
Lateral cortex anterior	15	15	16	12	0	0	0	0	0	0
Prelimbic cortex	9	8	7	12	5	0	0	0	4	1
Piriform cortex	1	0	2	5	12	12	2	16	16	21
Globus pallidus	14	8	13	7	0	0	0	0	0	0
Thalamic nuclei	16	15	15	16	13	5	0	0	0	0
Presubiculum	11	15	12	9	4	0	0	0	0	0
Postsubiculum	13	15	15	16	2	0	0	0	0	0
Hippocampus	16	16	15	16	9	0	0	1	8	4
Somatosensory cortex	0	0	0	0	0	0	0	0	0	1
Retrosplenial cortex	16	16	16	16	16	4	0	16	16	21
Mammillary nuclei	13	13	13	13	13	12	0	0	0	0
Lateral cortex middle/posterior	14	16	16	16	15	0	0	0	1	0
Frontal association cortex	12	13	12	11	2	0	0	0	0	0
Septal nuclei	6	4	4	1	3	0	0	0	1	4
Accumbens	14	15	15	13	0	0	0	0	0	0
Pontine nuclei	10	14	10	14	12	0	0	0	0	0
Motor cortex	16	15	16	13	0	0	0	0	0	0
Caudate putamen	15	15	14	10	0	0	0	0	0	0
Dentate	1	2	0	4	7	8	2	1	7	12
Hypothalamic nuclei	12	9	8	9	0	0	0	0	0	0
Amygdaloid nuclei	16	15	12	6	0	0	0	0	4	0
Dorsal peduncular nucleus	0	3	4	1	0	0	0	0	5	0
Dorsal peduncular cortex	7	2	1	6	1	0	0	0	0	4
Bed nuclei stria terminalis	0	0	0	2	1	0	0	0	0	0
Dorsal tenia tecta	6	9	1	0	0	0	0	0	3	0
Orbital cortex	1	0	2	2	0	0	0	0	0	0
Ventral pallidum	1	0	0	0	0	0	0	0	0	0
Claustrum	2	1	0	0	0	0	0	0	0	0
Cortex layers 1-4	0	0	0	0	0	0	0	0	4	0

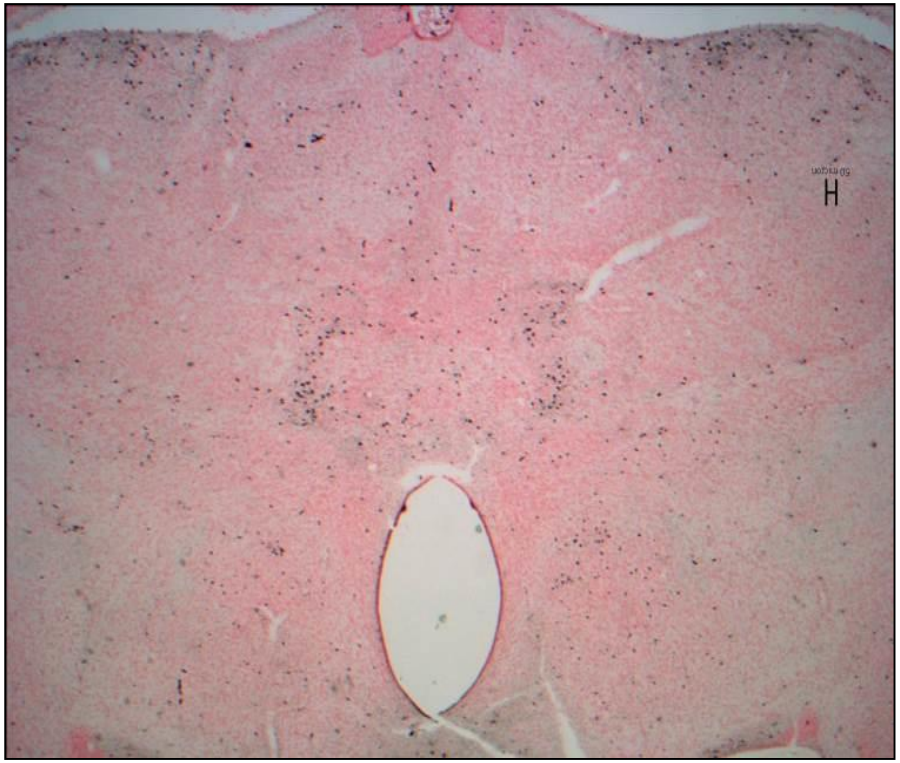
Sites at Which Degeneration Was Observed in MK-801 Male Rats using the Caspase Apoptosis stain

Age (Postnatal days)	8	9	10	12	14	17	24	40	71	113
No.Animals with Lesions	16	16	16	16	17	12	3	1	0	0
Number Examined	16	16	16	16	17	16	17	16	16	21
Olfactory lobe	9	7	10	13	8	2	1	0	0	0
Olfactory nucleus	0	0	0	1	1	0	1	0	0	0
Olfactory bulb	0	0	0	0	7	1	1	0	0	0
Entorhinal cortex	13	13	12	10	10	0	0	0	0	0
Cingulate cortex	15	15	16	9	7	0	0	0	0	0
Lateral cortex anterior	8	14	13	5	4	0	0	0	0	0
Prelimbic cortex	3	6	3	10	1	0	0	0	0	0
Piriform cortex	0	0	1	4	9	8	0	0	0	0
Globus pallidus	3	0	1	2	0	0	0	0	0	0
Thalamic nuclei	15	14	15	14	12	0	0	1	0	0
Presubiculum	2	7	8	6	1	0	0	0	0	0
Postsubiculum	14	15	15	14	0	0	0	0	0	0
Hippocampus	16	16	16	15	3	0	0	0	0	0
Retrosplenial cortex	15	16	16	16	17	4	0	0	0	0
Mammillary nuclei	2	0	3	7	16	10	0	0	0	0
Lateral cortex middle/posterior	8	16	16	14	0	0	0	0	0	0
Frontal association cortex	6	8	2	3	0	0	0	0	0	0
Septal nuclei	5	3	3	1	2	0	0	0	0	0
Accumbens	12	8	6	4	1	0	0	0	0	0
Pontine nuclei	1	6	6	10	10	5	0	1	0	0
Motor cortex	10	13	12	5	0	0	0	0	0	0
Caudate putamen	10	7	6	3	0	0	0	0	0	0
Hypothalamic nuclei	1	3	3	8	0	0	0	0	0	0
Amygdaloid nuclei	7	8	9	5	0	0	0	0	0	0
Dorsal peduncular nucleus	3	3	0	0	0	0	0	0	0	0
Dorsal peduncular cortex	0	0	0	2	0	0	0	0	0	0
Bed nuclei stria terminalis	0	0	0	1	0	0	0	0	0	0
Dorsal tenia tecta	2	4	1	0	0	0	0	0	0	0
Orbital cortex	0	0	0	1	1	0	0	0	0	0
Visual cortex	0	0	0	0	1	0	0	0	0	0
Corpus callosum	1	0	1	0	0	0	0	0	0	0
Hypothalamus	0	0	0	0	2	0	0	0	0	0

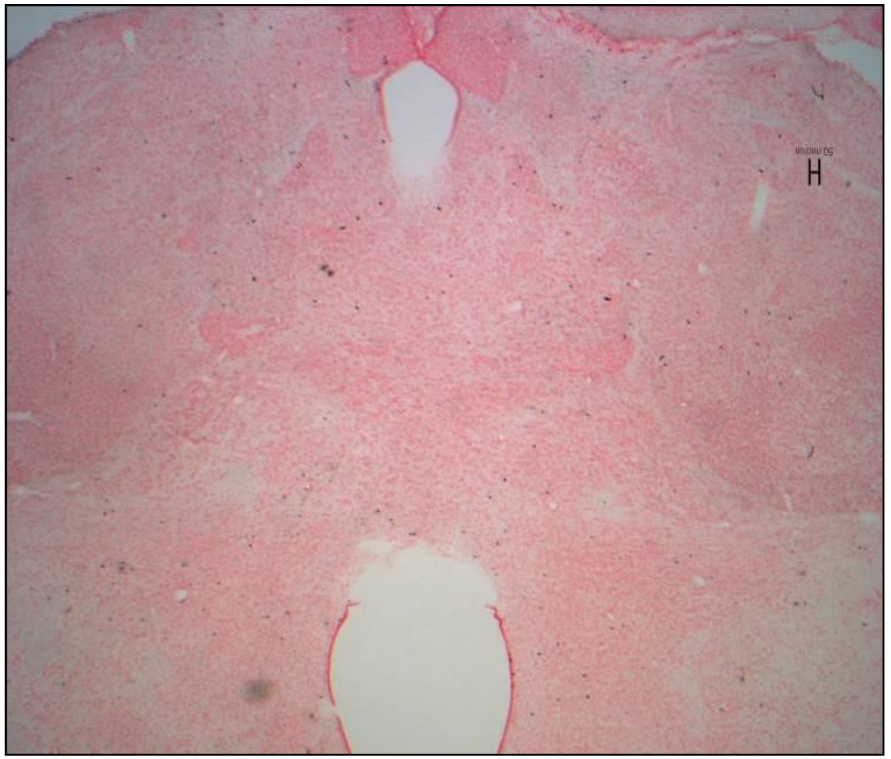
Table 4: Sites at Which Degeneration Was Observed in MK-801 Female Rats using the Caspase Apoptosis stain

Age (Postnatal days)	8	9	10	12	14	17	24	40	71	113
No.Animals with Lesions	16	15	16	16	16	15	11	11	16	21
Number Examined	16	16	16	16	16	16	18	16	16	21
Olfactory lobe	7	6	9	11	8	3	0	6	0	0
Olfactory bulb	0	0	0	0	7	2	10	4	0	0
Entorhinal cortex	10	10	12	9	13	0	0	0	0	0
Cingulate cortex	14	13	15	11	9	0	0	0	0	0
Lateral cortex anterior	8	14	14	2	0	0	0	0	0	0
Prelimbic cortex	2	2	5	6	3	0	0	0	0	0
Infralimbic/prelimbic cortex	0	0	0	0	1	0	0	0	0	0
Piriform cortex	0	0	2	2	10	9	4	7	0	0
Globus pallidus	0	0	5	1	0	0	0	0	0	0
Thalamic nuclei	15	11	16	12	14	0	0	0	0	0
Presubiculum	5	7	11	3	0	0	0	0	0	0
Postsubiculum	14	15	16	10	3	0	0	0	0	0
Hippocampus	15	15	16	13	7	0	0	0	0	0
Retrosplenial cortex	15	11	16	15	15	3	0	0	0	0
Mammillary nuclei	3	2	5	8	13	12	0	0	0	0
Lateral cortex middle/posterior	12	15	16	13	2	0	0	0	0	0
Frontal association cortex	4	4	4	3	0	0	0	0	0	0
Septal nuclei	1	2	2	0	1	0	0	0	0	0
Accumbens	4	9	7	2	1	0	0	0	0	0
Pontine nuclei	2	6	3	10	10	3	0	0	0	0
Motor cortex	10	11	14	5	0	0	0	0	0	0
Caudate putamen	4	3	6	3	0	0	0	0	0	0
Dentate	0	0	0	0	0	1	0	0	0	0
Hypothalamic nuclei	0	0	1	4	4	0	0	0	0	0
Amygdaloid nuclei	7	5	12	3	0	0	0	0	0	0
Dorsal peduncular nucleus	0	0	0	0	0	0	0	0	0	0
Dorsal peduncular cortex	1	2	0	0	0	0	0	0	0	0
Bed nuclei stria terminalis	0	0	0	0	0	0	0	0	0	0
Dorsal tenia tecta	1	5	0	0	0	0	0	0	0	0
Orbital cortex	0	1	0	0	1	0	0	0	0	0
Visual cortex	0	0	0	0	1	0	0	0	0	0
Corpus callosum	2	0	1	0	0	0	0	0	0	0
Subiculum	0	0	0	0	2	0	0	0	0	0
Anterior commissure	0	0	1	0	0	0	0	0	0	0
Fimbria hippocampus	1	0	0	0	0	0	0	0	0	0

Thalamic Nuclei, PND 8, AmCuAg Degeneration stain

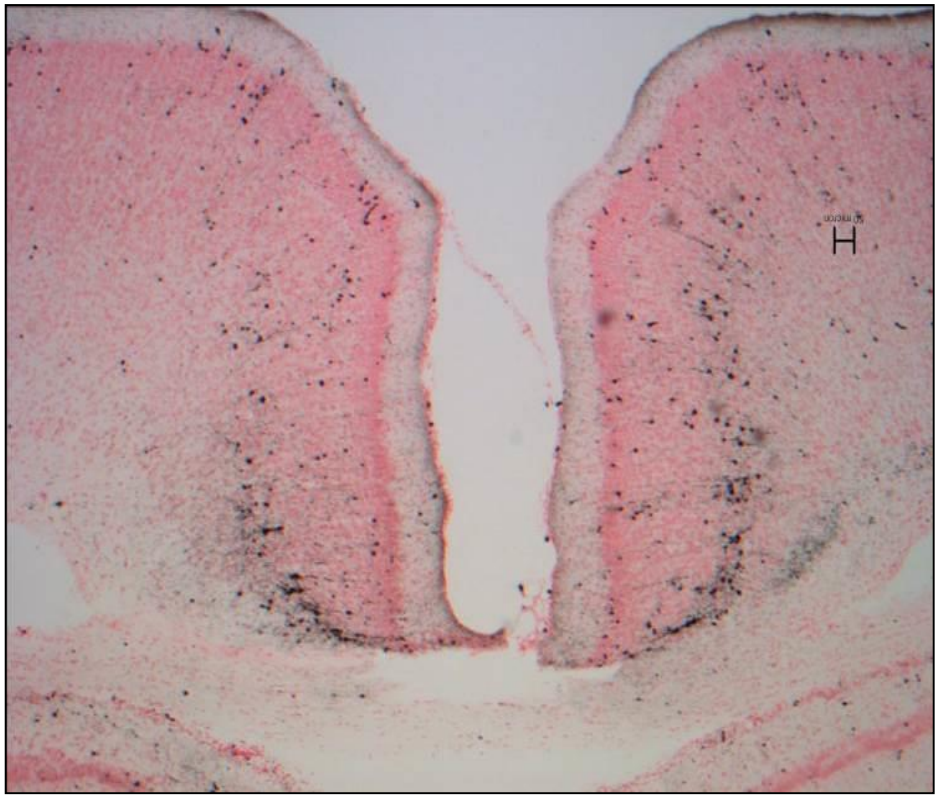


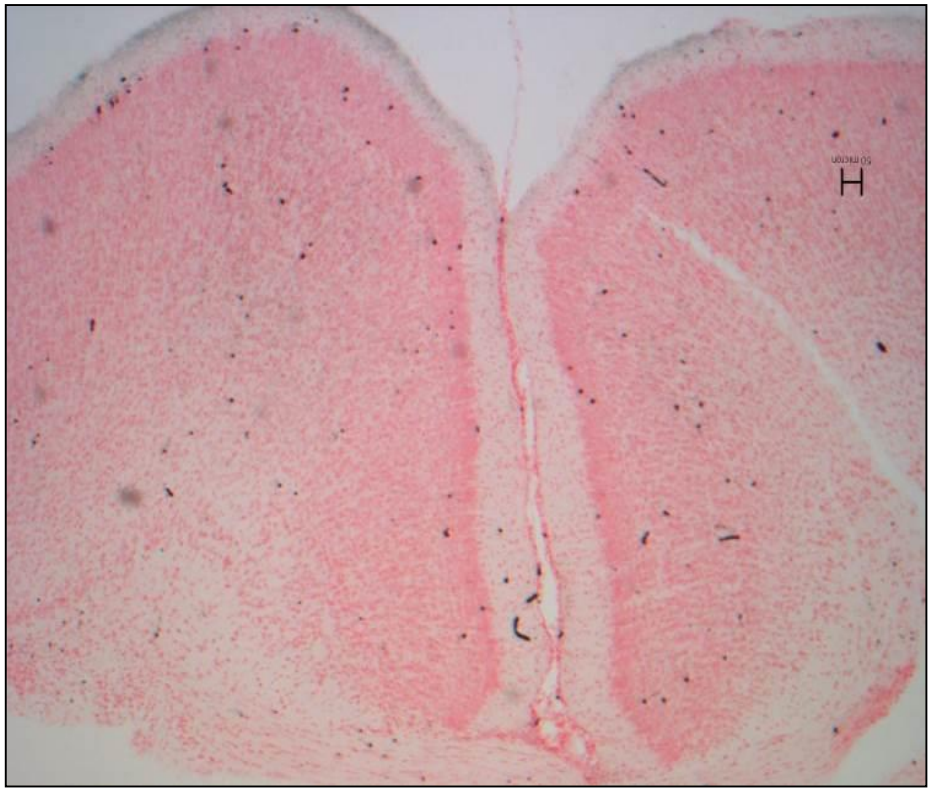
MK-801; Note greater number of Thalamic Nuclei involved as compared to PND 14. Calibration mark = 50μ



Control; Same thalamic nuclei as shown in MK-801. Black dots are RBCs in capillaries. Calibration mark = 50μ

Retrosplenial cortex, PND 8, AmCuAg Degeneration stain

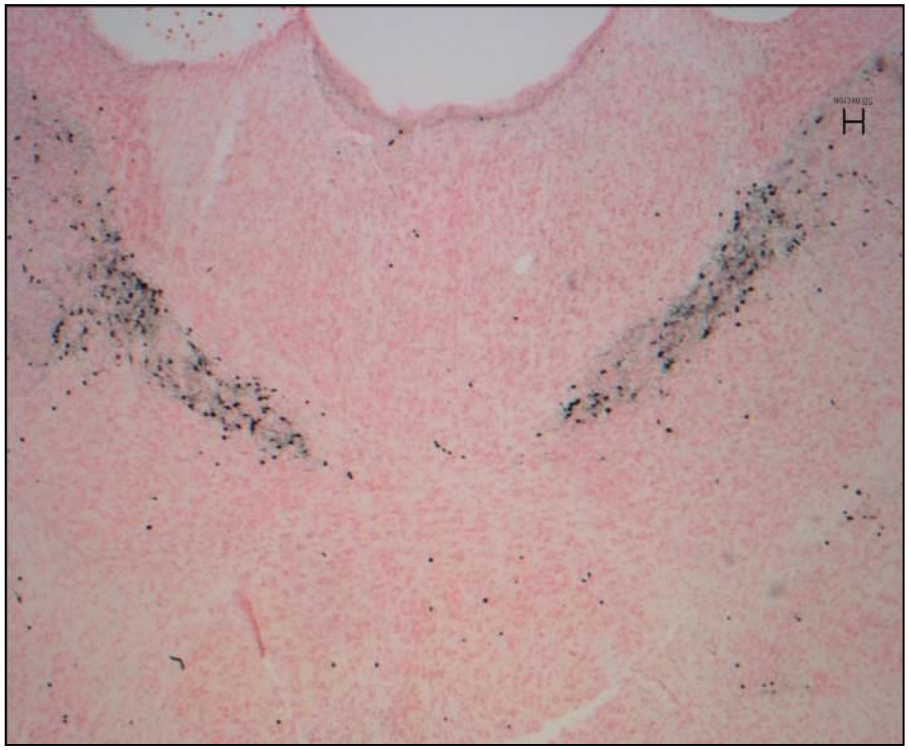




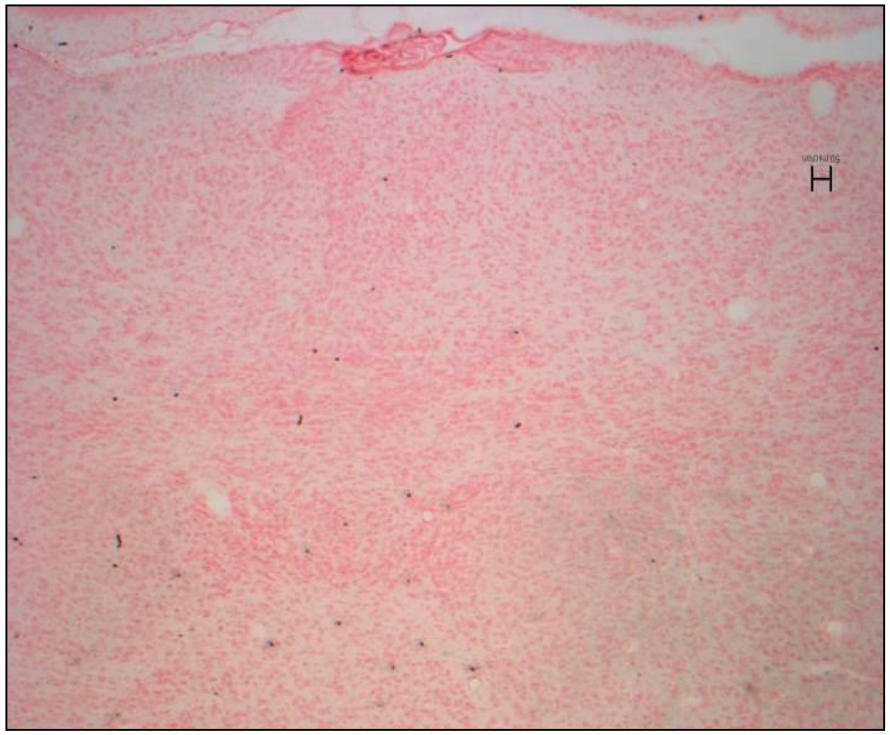
MK-801; Retrosplenial cortex,
Calibration mark = 50μ

Control; Same retrosplenial cortex region
Black dots are RBCs in capillaries.
Calibration mark = 50μ

Thalamic nuclei, PND 14, AmCuAg Degeneration stain

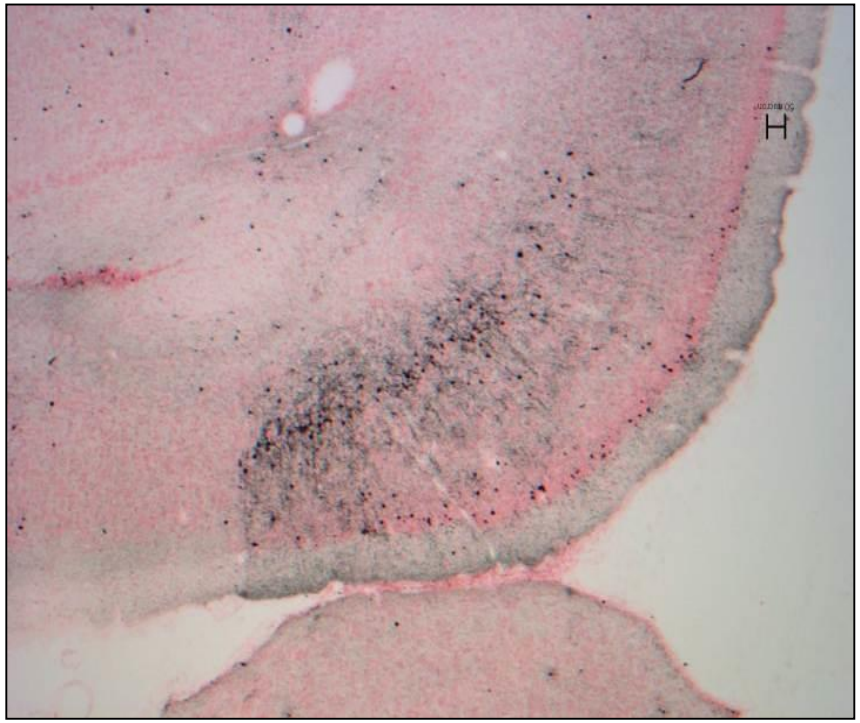


MK-801; thalamic nuclei
Calibration mark = 50μ

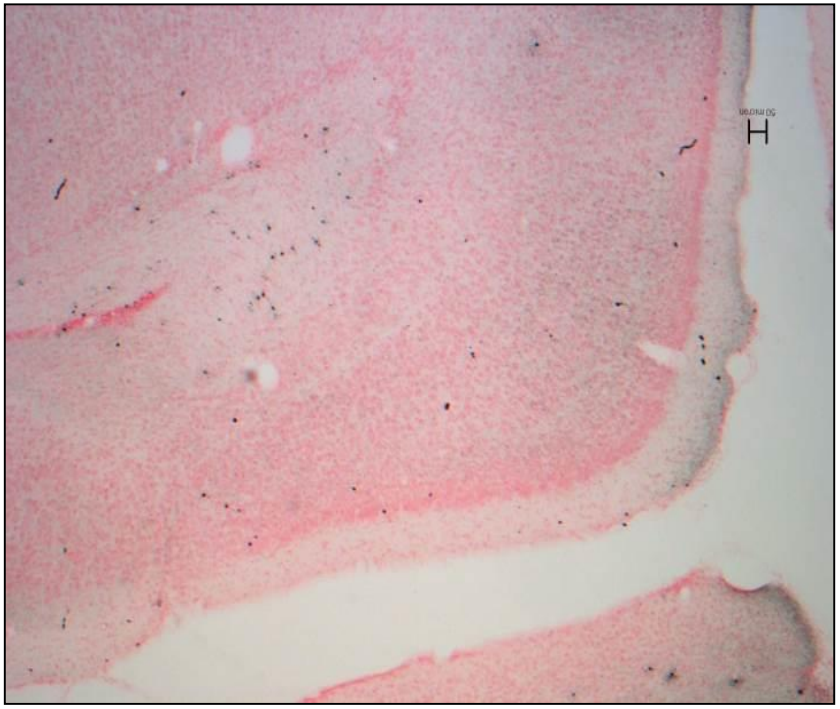


Control; Same thalamic nuc
lei. Black dots are RBCs in capillaries.
Calibration mark = 50μ

Retrosplenial cortex, PND 14, AmCuAg Degeneration stain

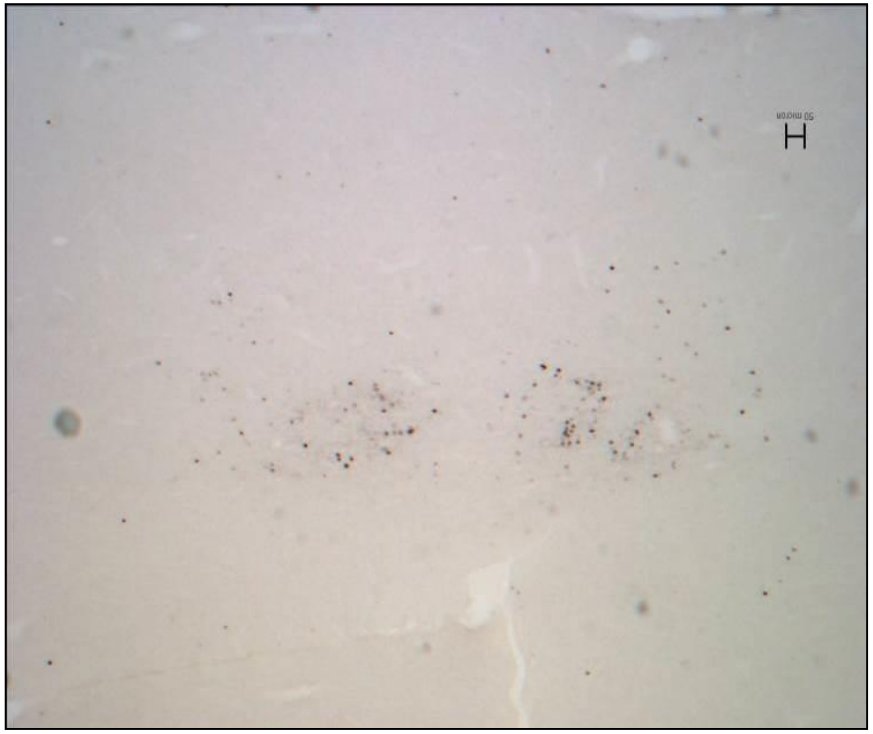


MK-801; Retrosplenial cortex..
Calibration mark = 50 μ

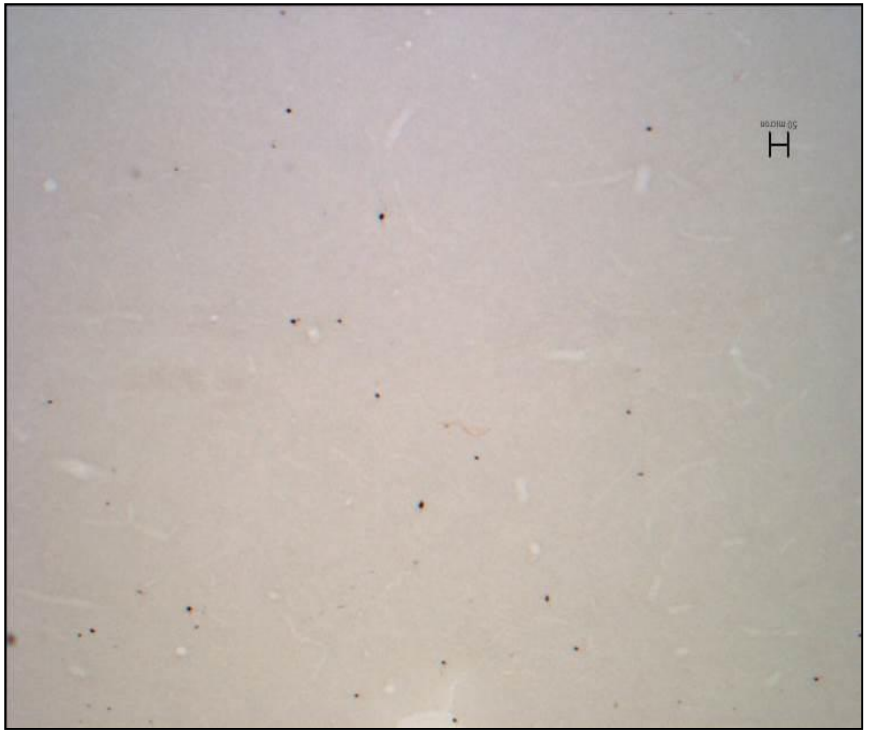


Control; Same retrosplenial cortex region. Black dots are RBCs in capillaries.
Calibration mark = 50 μ

Thalamic nuclei, PND 8, Caspase-9 stain

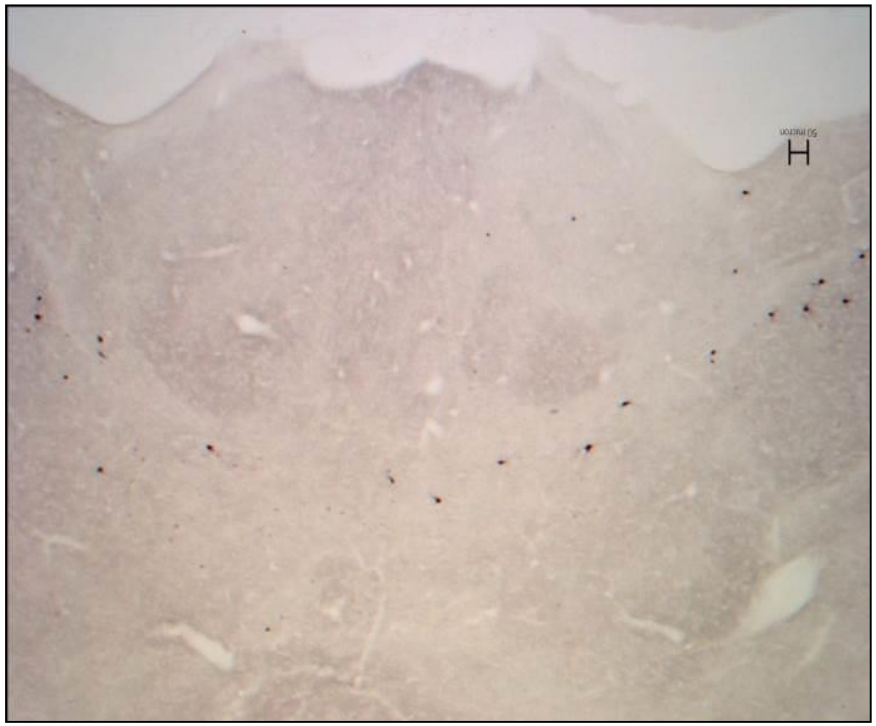


MK-801. Note extent of involvement as compared to degeneration stain (Fig4a).
Calibration mark = 50 micron

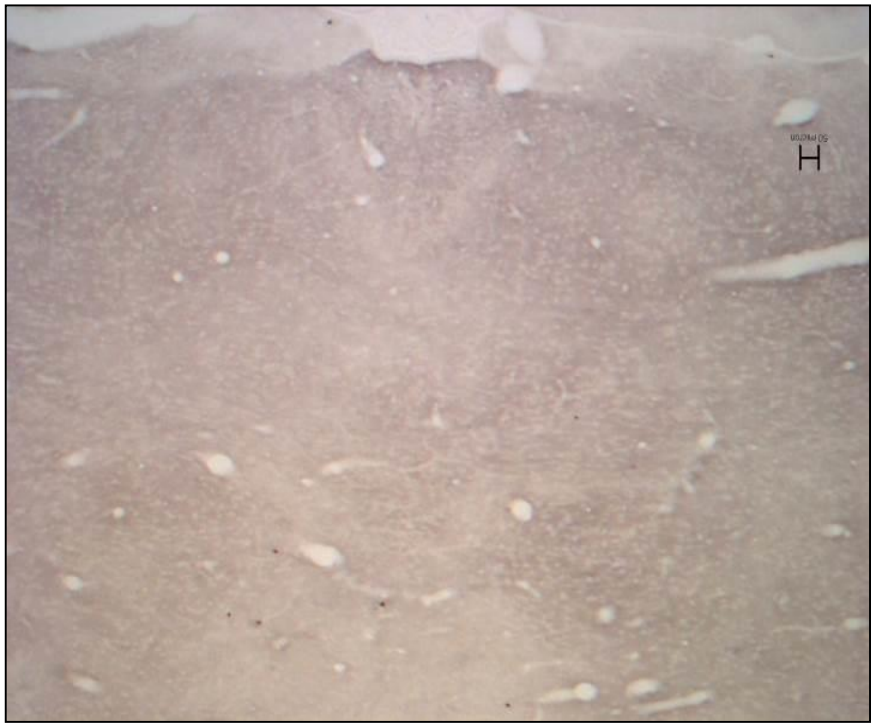


Control; Same thalamic area as shown in Fig 7a Black dots represent background staining.
Calibration mark = 50 micron

Thalamic nuclei, PND 14, Caspase stain



MK-801; Same thalamic nuclei
Calibration mark = 50μ



Control; Same thalamic area.
Calibration mark = 50 μ

Sites at Which Degeneration Was Observed in Vehicle Control Rats

Degeneration and to a greater extent, apoptosis, were present in numerous brain sites of males and females from vehicle control groups at earlier PNDs, most prominently from PND 8 through PND 24.

Males	PND	Incidence	Severity	
Site			Minimal	Mild
Accumbens	9	2/10	1/10	1/10
Pontine nuclei	9	2/10	0/10	1/10
Piriform cortex	14	1/10	1/10	0/10
Dentate	17	6/10	6/10	0/10
Piriform cortex	24	10/10	5/10	5/10
Females	PND	Incidence	Severity	
Site			Minimal	Mild
Accumbens	9	8/10	8/10	0/10
Entorhinal cortex	9	1/10	1/10	0/10
Lateral cortex middle/posterior	9	1/10	1/10	0/10
Caudate Putamen	9	1/10	1/10	0/10
Caudate Putamen	10	1/10	0/10	1/10
Thalamic nuclei	10	1/10	0/10	1/10
Corpus callosum	10	1/10	0/10	1/10
Dentate	17	8/10	7/10	1/10
Piriform cortex	24	9/9	3/9	6/9

Bolon, B. et al. 2013 STP Position Paper: Recommended practices for sampling and processing the nervous system (brain, spinal cord, nerve and eye) during nonclinical general toxicity studies. Toxicol Pathol 41:7, 1028-48.

Trimming the adult rodent brain. (Bolon, B., et al., 2013)

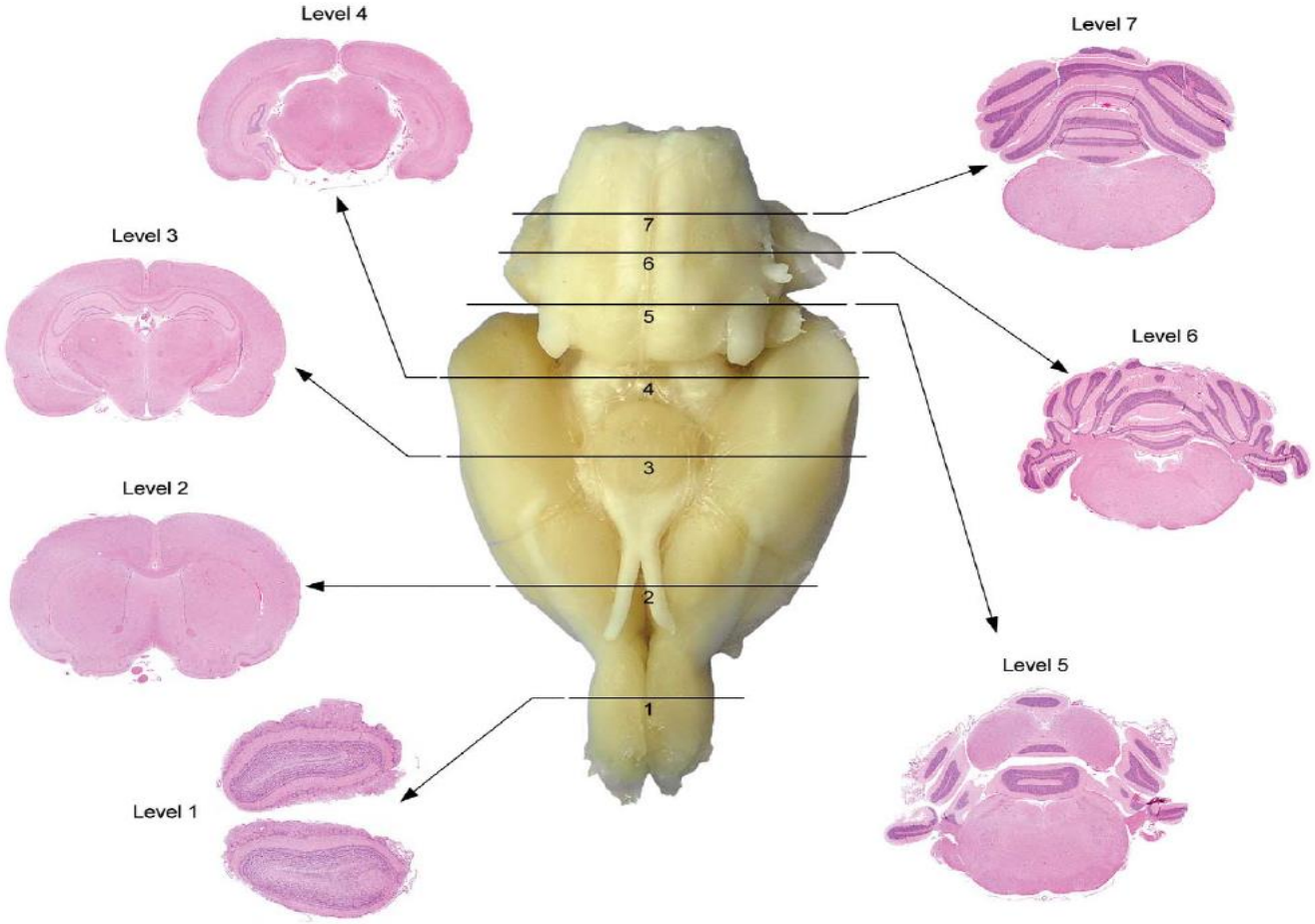


FIGURE 1.—Trimming the adult rodent brain. Representation of external landmarks on the ventral surface of the adult rat brain (central whole-organ image) used to consistently attain coronal sections with generally similar internal structures (peripheral H&E-stained sections) for neuropathology assessment during general toxicity studies. The solid black lines show where to place transverse cuts, and the black arrows point to the sections produced by trimming the brain in this manner. (*Note*: These trimming planes are used as an example. They are comparable to but not exactly identical to those demonstrated for the 7-level brain sampling scheme adopted recently by the U.S. National Toxicology Program [Rao et al. 2011]. Such differences are indicative of the modest variations in orientation and positioning of the brain sampling levels that should be expected among animals within a single study, across multiple studies, and among institutions.)

Major landmarks for level orientation during brain sampling in GLP-type nonclinical general toxicity studies in adult rodents.

Levels							
1	2	3	4	5	6	7	Brain structures (listed from rostral to caudal)
X							Olfactory bulb
	X						Anterior commissure
	X						Septal nuclei
	X						Caudate/putamen
	X	X	X				Cerebral cortex (frontal, parietal, temporal, occipital)
	X	X					Corpus callosum
	X	X					Internal capsule
	X	X					External capsule
		X					Optic tract
		X					Amygdala
		X	X				Hippocampus
		X					Thalamus
		X					Hypothalamus
		X	X				Cerebral peduncles
			X				Midbrain, rostral
				X			Midbrain, caudal
				X			Pons
				X	X	X	Pyramids
				X	X	X	Cerebellum
					X		Deep cerebellar nuclei
			X	X	X	X	Reticular formation
					X	X	Trigeminal nuclei and tracts
						X	Medulla oblongata
		X				X	Choroid plexus

Take Away Message

- Juvenile Toxicity Studies are designed & performed on a case-by-case basis (no standardized study protocols).

- Study design of Juvenile Toxicity Studies needs to cover phases of growth & development of organ systems at risk in the pediatric population.

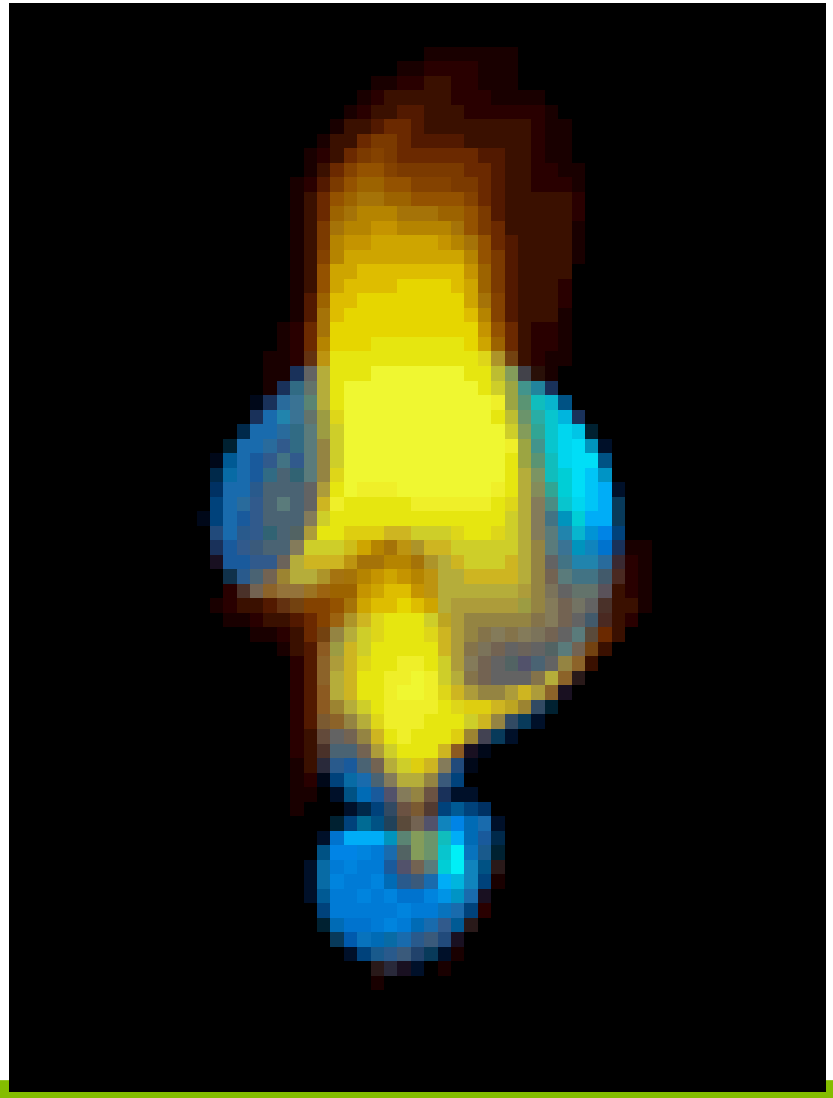
- Rat is 1st choice species and will involve direct dosing of pups. Pathologists must understand ‘normal’ organ development.

- Do your homework. Each study is unique and requires intellectual participation of numerous technical and scientific personnel.

About the Speaker

- Name: Robert M. Parker, PhD, DABT
- Education:
  - BS: Zoology (San Diego State University, San Diego CA)
  - MS: Anatomy (School of Veterinary Medicine, University of California, Davis CA)
  - PhD: Anatomy (School of Medicine, University of California, Davis CA)
- Interests: Developmental and Reproductive Toxicology, Neurotoxicology, Neurobehavior, Juvenile animal studies, Football, Baseball and Golf
- Career Path:
  - Assistant Professor of Anatomy (College of Osteopathic Medicine of the Pacific)
  - Study Director and Head, DART Section (Pathology Associates, Inc., National Center for Toxicological Research)
  - Manager of Toxicology and Study Director (Transgenic Sciences Inc., Redfield Laboratories)
  - Associate Director of Neurotoxicology (Primedica Argus Research Laboratories)
  - Director, Developmental, Reproductive and Neurological Toxicology (DuPont Haskell Laboratory for Toxicology and Industrial Medicine)
  - Research Leader, Toxicologist (Hoffmann-LaRoche, Inc.)
  - Director, Developmental and Reproductive Toxicology (Huntingdon Life Sciences)

Any Burning Questions?



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Comparative Organ System Development Literature

Birth Defects Research (Part B) Volume 68:

- Bone development
- Renal development
- Lung development
- Male reproductive system
- Female reproductive system
- Heart development
- Immune system development
- CNS development

Birth Defects Research (Part B) Volume 74:

- Gastrointestinal development

Birth Defects Research (Part B) Volume 77:

- Postnatal growth and morphological development of the brain

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Dentate	17	6/10	6/10	0/10
Piriform cortex	24	10/10	5/10	5/10
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Lateral cortex middle/posterior	9	1/10	1/10	0/10
Caudate Putamen	9	1/10	1/10	0/10
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Thalamic nuclei	10	1/10	0/10	1/10
Corpus callosum	10	1/10	0/10	1/10
Dentate	17	8/10	7/10	1/10
Piriform cortex	24	9/9	3/9	6/9

Juvenile Toxicology Literature

Birth Defects Research (Part B) Volume 92:

- **Introduction to special issue on the value of juvenile animal studies**
- **The value of juvenile animal studies: a pediatric clinical perspective**
- **Juvenile animal studies and pediatric drug development: a European regulatory perspective**
- **Juvenile animal studies and pediatric drug development retrospective review: use in regulatory decisions and labeling**
- **The value of juvenile animal studies: a Japanese industry perspective**
- **Nonclinical support of pediatric drug development in a global context: an industry perspective**
- **The value of juvenile animal studies "What have we learned from preclinical juvenile toxicity studies? II"**
- **Value of juvenile animal studies**

DART and Juvenile Toxicology

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- Histopathology of Preclinical Toxicity Studies: Interpretation and Relevance in Drug Safety Evaluation. Fourth Edition, 2012 Peter Greaves (Ed.), Academic Press, Elsevier, San Diego, CA

Developmental and Reproductive Toxicology Literature

Birth Defects Research (Part B) Volume 86:

- **The Nonclinical Fertility Study Design for Pharmaceuticals.**
- **Embryo-Fetal Developmental Toxicity Study Design for Pharmaceuticals.**
- **Pre- and Postnatal Developmental Toxicity Study ; Design for Pharmaceuticals.**
- **Juvenile Animal Toxicity Study Designs to Support Pediatric Drug Development.**