Neuropathology Assessments in Toxicology Studies: Challenges and Principles

Society of Toxicologic Pathology – India (STP-I)

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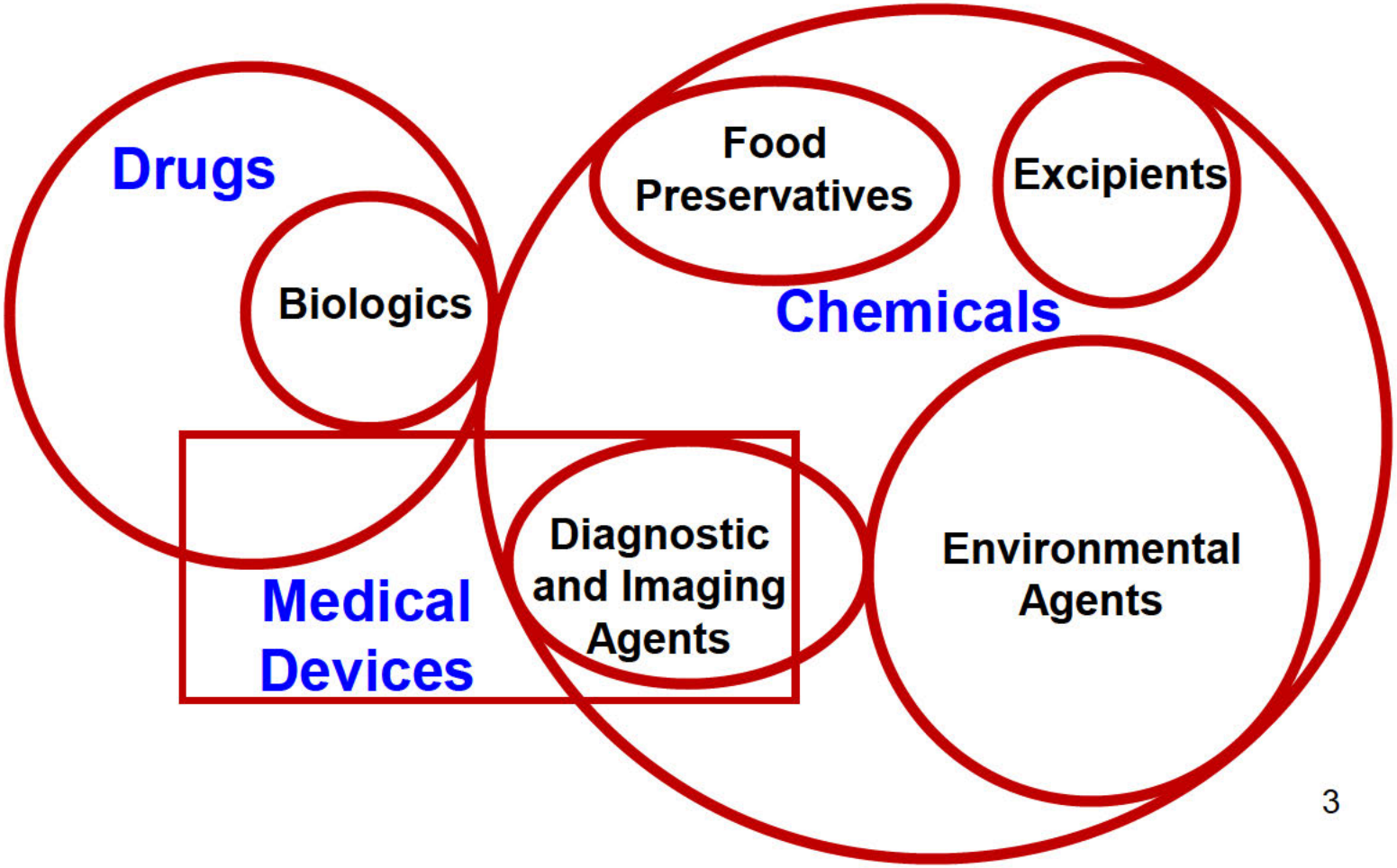
US Food and Drug Administration/CDER

Division of Psychiatric Products

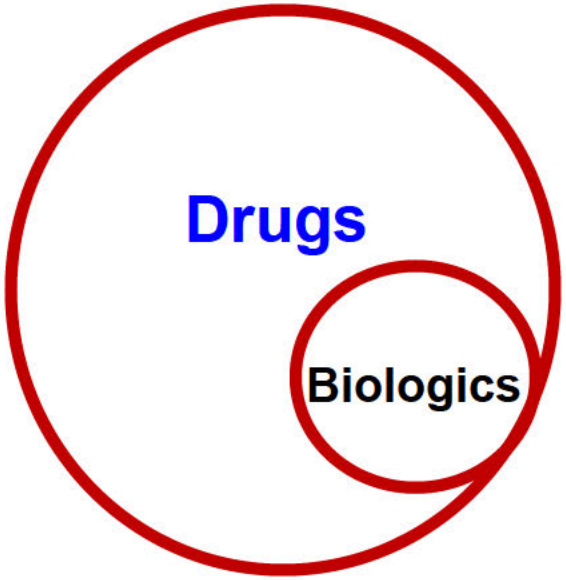
Disclaimer

The opinions expressed in this presentation are my own and do not reflect official support or endorsement by the Food and Drug Administration

Neuropathology Assessments in Toxicology Studies

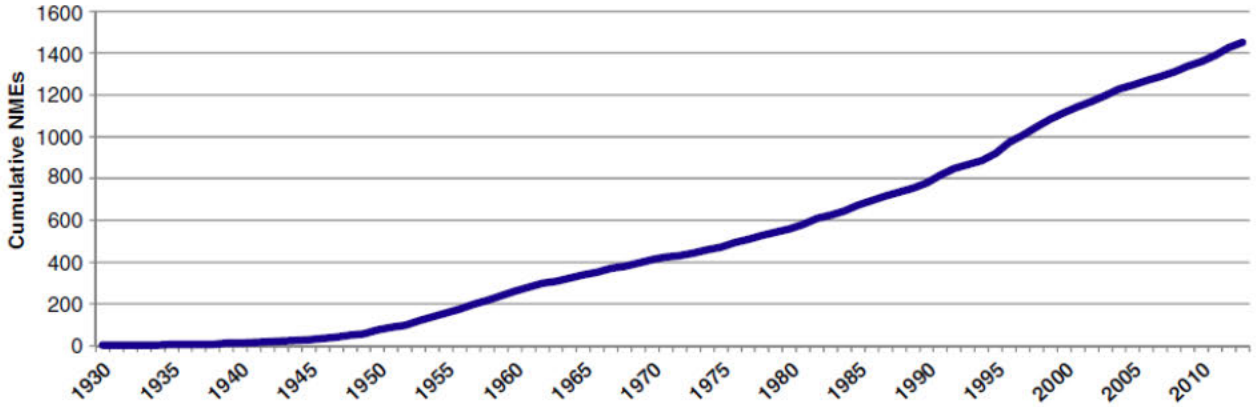


Drugs – how many ?



How many drugs exist ?

1453 FDA-approved New Molecular Entities (NMEs) between 1827 -2013



Kinch et al, 2014

Drugs – how many are neurotoxic ?

Drug Information Journal, Vol. 35, pp. 293–317, 2001
Printed in the USA.

0092-8615/2001

EVALUATION OF THE CHARACTERISTICS OF SAFETY WITHDRAWAL OF PRESCRIPTION DRUGS FROM WORLDWIDE PHARMACEUTICAL MARKETS–1960 TO 1999*

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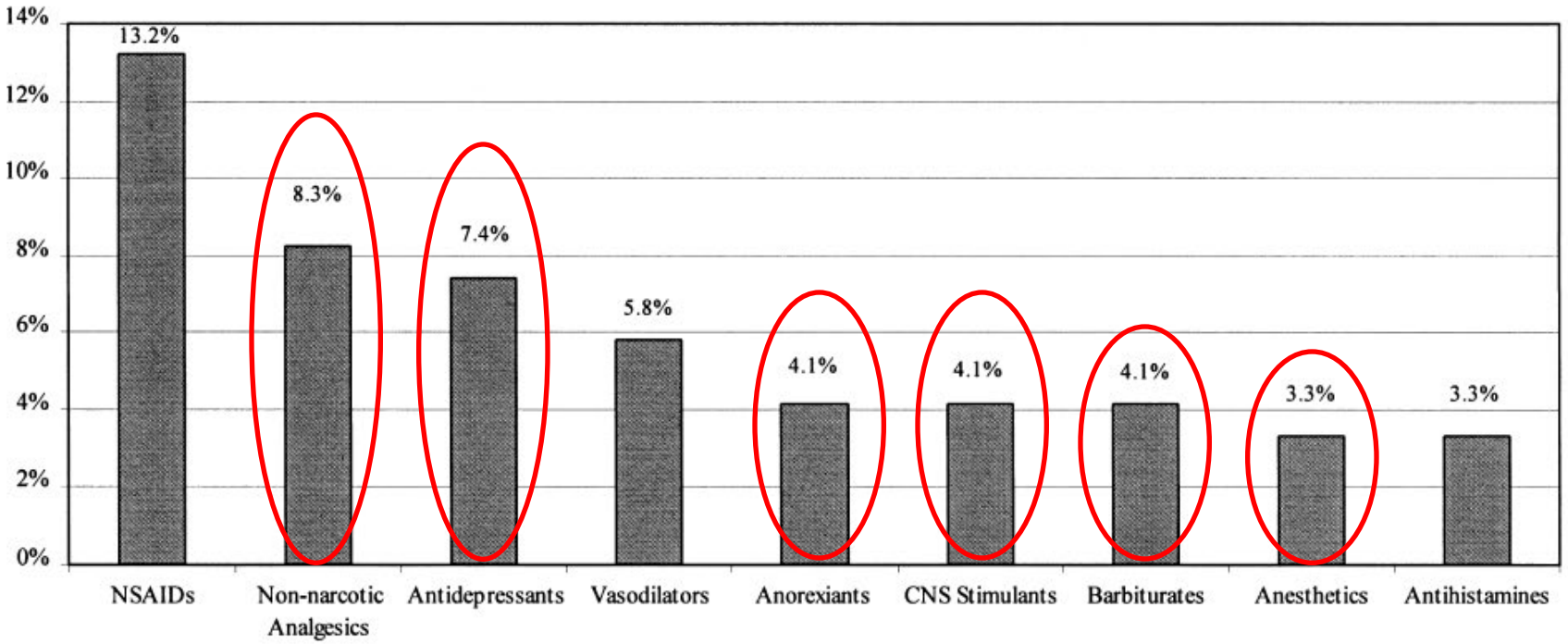

FIGURE 1. Most common classes of drugs withdrawn.

Fung et al, 2001

E-appendix Table 1: List of medicinal products withdrawn because of adverse drug reactions.

Medicinal product	Class	Mechanism of action	Therapeutic indication	Launch date	Year of first ADR report	Year first withdrawn	Countries withdrawn	Reason for withdrawal	Level of evidence†
Budipine	Antiparkinsonian	Muscarinic & NMDA receptor antagonist	Parkinson's	1979	2000	2000	Germany	Cardiovascular	4
Bufexamac	Analgesic	COX-1 & COX-2 inhibition	Analgesia	1973	1973	1990	France	Skin	4
Buflomedil‡	Vasodilator	α-adrenergic blockade	Peripheral arterial occlusive disease	1970	1981	2006	France, Europe	Neurotoxicity; cardiotoxicity	4
Buformin‡	Hypoglycemic	Reduce gluconeogenesis	Diabetes	1950	1969	1978	Germany, Austria, Belgium, Ireland	Metabolism	4
Bumadizone injection	Analgesic	COX-1 & COX-2 inhibition	Rheumatism	1972	1978	1986	Oman	Hematologic	5*
Bunamiodyl‡	Radiography	Selective secretion in bile	Radiography	1958	1962	1964	USA, Sweden, Venezuela	Kidney	4
Buprenorphine‡	Analgesic	Agonist–antagonist opioid receptor modulator	Analgesia	1978	1983	1986	Egypt	Fatalities (IV use)	4
Bupropion	Antidepressant	Norepinephrine-dopamine reuptake inhibitor	Depression	1985	1985	1986	USA	Nervous system	4
Butamben	Anesthetic	↓ neuronal membrane permeability to sodium ions	Local anesthesia	1923	1947	1964	UK	Allergic, psychiatric, skin	4
Cadralazine	Antihypertensive	Peripheral arteriolar vasodilator	Hypertension	1989	1991	1992	Norway	Immunologic	2
Camazepam	Sedative-hypnotic	GABA-A receptor modulation	Hypnosedation	1978	1984	1984	Netherlands	Immunologic	4
Canrenone	Antihypertensive	Diuresis, aldosterone antagonist	Aldosteronism, CHF, hypertension	1966	1976	1986	Germany	Tumorigenicity	5*
Carbinoxamine	Antihistamine	Competes with free histamine for binding at HA-receptor sites	Allergy	1953	1987	2008	Iraq	Neurotoxicity	4
Carisoprodol	Muscle relaxant	Unknown	Sprain, muscle injury	1959	2002	2007	Sweden, EU, Indonesia	Abuse	4
Cartilage + bone marrow	Antiarthritic		Degenerative joint disease	1960	1989	1992	Germany	Skin	3
Catechic extract‡	Antiinflammatory	Unclear	Benign prostatic hyperplasia	1972	1979	1982	France	Hematologic	4

Onakpoya et al, 2016

Toxicity Type	# of Drugs Withdrawn
Neurotoxicity/Nervous System	53
Psychiatric	18
Drug Abuse	35
Sensory Systems	19

FDA notice on 6/16/2009

FDA Advises Consumers Not To Use Certain Zicam Cold Remedies
Intranasal Zinc Product Linked to Loss of Sense of Smell

The U.S. Food and Drug Administration today advised consumers to stop using three products marketed over-the-counter as cold remedies because they are associated with the loss of sense of smell (anosmia). Anosmia may be long-lasting or permanent.

The products are:
--Zicam Cold Remedy Nasal Gel
--Zicam Cold Remedy Nasal Swabs
--Zicam Cold Remedy Swabs, Kids Size (a discontinued product)

The FDA has received more than 130 reports of loss of sense of smell associated with the use of these three Zicam products. In these reports, many people who experienced a loss of smell said the condition occurred with the first dose; others reported a loss of the sense of smell after multiple uses of the products.

"Loss of sense of smell is a serious risk for people who use these products for relief from cold symptoms," said Janet Woodcock, M.D., director of the FDA's Center for Drug Evaluation and Research (CDER). "We are concerned that consumers may unknowingly use a product that could cause serious harm, and therefore we are advising them not to use these products for any reason."

People who have experienced a loss of sense of smell or other problems after use of the affected Zicam products should contact their health care professional. The loss of sense of smell can adversely affect a person's quality of life, and can limit the ability to detect the smell of gas or smoke or other signs of danger in the environment.

The FDA has issued Matrixx Initiatives, maker of these Zicam products, a warning letter telling it that these products cannot be marketed without FDA approval.

"Companies have an obligation to the public to demonstrate to the FDA that their products are safe, particularly when there is evidence they may be causing serious adverse events, and they are marketed for minor, self-limiting conditions like the common cold," said Deborah M. Autor, director of CDER's Office of Compliance.

http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2009/ucm167065.htm

Slide Courtesy: Drs. Patel and Whittaker, FDA-CDER

OPEN ACCESS Freely available online

PLoS one

Zicam-Induced Damage to Mouse and Human Nasal Tissue

Jae H. Lim[1], Greg E. Davis[1], Zhenshan Wang[2], Vicky Li[2], Yuping Wu[2], Tessa C. Rue[3], Daniel R. Storm[2]*

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Abstract

Intranasal medications are used to treat various nasal disorders. However, their effects on olfaction remain unknown. Zicam (zinc gluconate; Matrixx Initiatives, Inc), a homeopathic substance marketed to alleviate cold symptoms, has been implicated in olfactory dysfunction. Here, we investigated Zicam and several common intranasal agents for their effects on olfactory function. Zicam was the only substance that showed significant cytotoxicity in both mouse and human nasal tissue. Specifically, Zicam-treated mice had disrupted sensitivity of olfactory sensory neurons to odorant stimulation and were unable to detect novel odorants in behavioral testing. These findings were long-term as no recovery of function was observed after two months. Finally, human nasal explants treated with Zicam displayed significantly elevated extracellular lactate dehydrogenase levels compared to saline-treated controls, suggesting severe necrosis that was confirmed on histology. Our results demonstrate that Zicam use could irreversibly damage mouse and human nasal tissue and may lead to significant smell dysfunction.

Citation: Lim JH, Davis GE, Wang Z, Li V, Wu Y, et al. (2009) Zicam-Induced Damage to Mouse and Human Nasal Tissue. PLoS ONE 4(10): e7647. doi:10.1371/journal.pone.0007647

Editor: Hiroaki Matsunami, Duke Unviersity, United States of America

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Competing Interests: The authors have declared that no competing interests exist.

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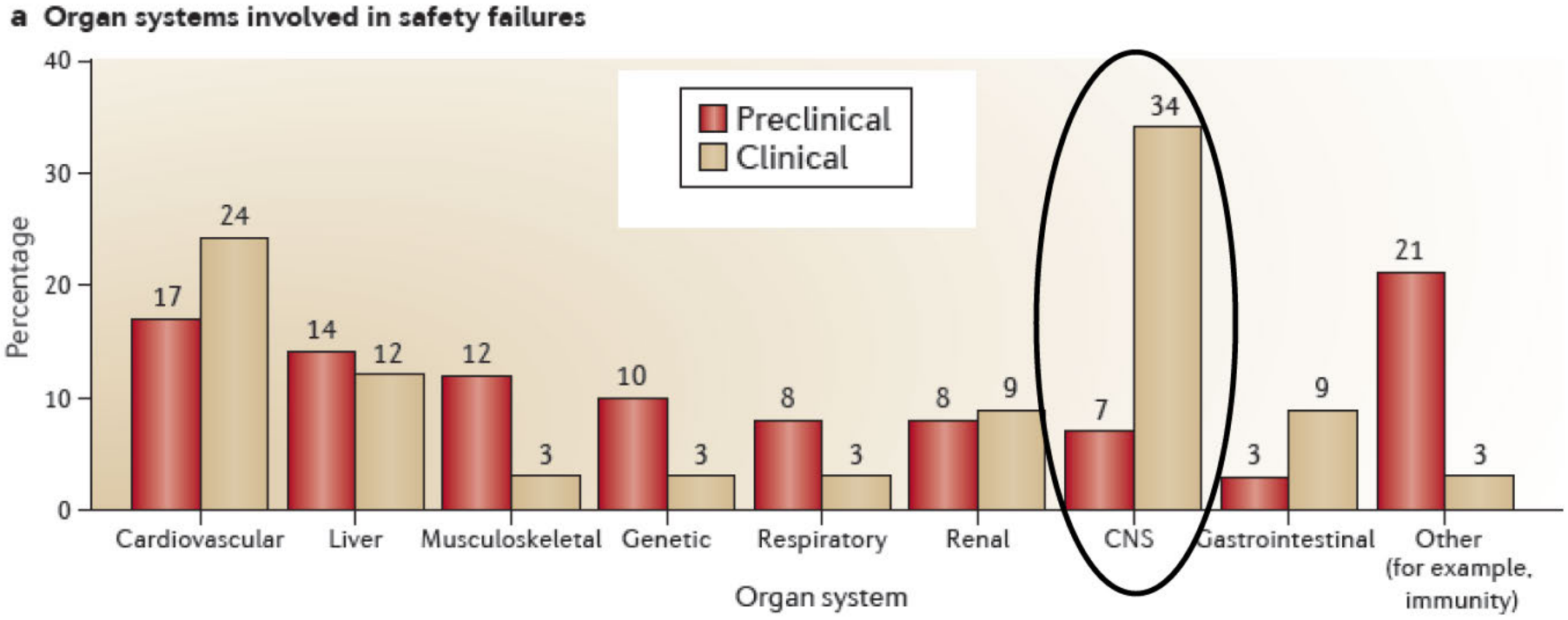
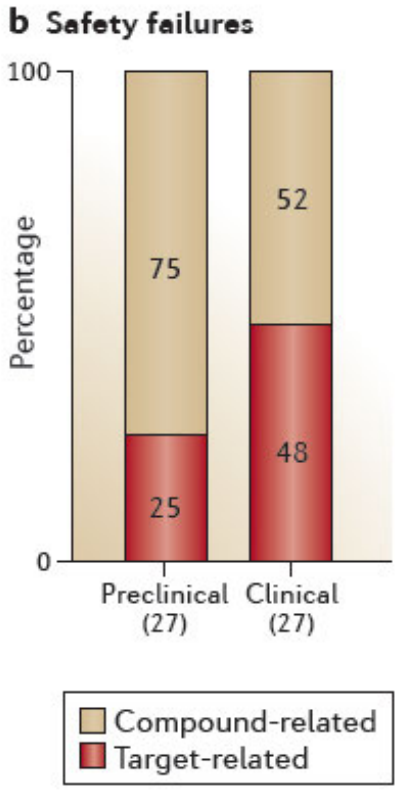


Cook et al, 2014.



Cook et al, 2014: "…75% of safety closures were compound-related (that is, they were **off-target** or other properties of the compound other than its action at the primary pharmacological target)…"

Cook et al, 2014, Nature Reviews, Drug Discovery, Volume 13, 2014, 419-431

Neurologic HTs were the most common category (22%) and they occurred disproportionately more frequently for neurologic drugs. It was noted that whereas the correlation with animal studies was independent of the therapeutic class of drug, these HTs led to termination of only 17% of neurologic drugs but to 45% of nonneurologic drugs. Such HTs are apparently more acceptable in neurologic drugs, this obviously depending to some extent on the concurrent therapeutic benefit. Correlations with findings in animals were much better for nonrodents than for rodents. The cases in rodents were all peripheral neuropathies in anticancer agents.

Olson et al, 2000, Reg Toxicol and Pharmacol, Volume 32, 2000, 56-67

Excipients in Generic Drugs

TABLE 1. Some of the excipients encountered in pharmaceutical drug products and associated potential toxicities[a]

Excipient	Function[b]	Toxicity ascribed[c]	ADI[d] (mg/kg Body weight)	Reference
Aspartame	Sweetening agent	GI, Phenylketonuria	40	(Golightly *et al.*, 1988)
Benzyl alcohol	Anti-microbial preservative	Respiratory, CNS	5	(Rowe *et al.*, 2009)
β-cyclodextrin	Stabilizing/Solubilizing agent	Renal	NA	(Frank *et al.*, 1976)
Chlorhexidine	Anti-microbial preservative	Anaphylactic reactions	NA	(Lockhart and Harle, 2001)
Diethylene glycol (DEG)	Solvent	Renal[e]	NA	(Rossoff, 2002)
Ethanol	Solvent	CNS	NA	(Winek, 2007)
Lanolin	Emulsifying agent	Dermal, hypersensitivity	NA	(Rowe *et al.*, 2009)
Magnesium silicate	Glidant/anticaking agent	Renal calculi	NA	(Levison *et al.*, 1982)
Mannitol	Diluent/sweetening agent	Hypersensitivity, Laxative effects	NA	(McNeill, 1985)
Polyethylene glycol	Ointment base, plasticizer, solvent	Hypersensitivity, Renal, Metabolic acidosis	10	(Fisher, 1978)
Propylene glycol	Solvent, preservative, stabilizing agent	Cardio toxicity, CNS[e]	25	(Arulanantham and Genel, 1978; Kapitein *et al.*, 2014)
Sodium metabisulfite	Antioxidant	Hypersensitivity, GI, CNS	7	(Baker *et al.*, 1981; Lester 1995)

GI, gastro intestinal system; CNS, central nervous system.
[a]The molecules listed here are generally encountered in pharmaceutical products; however, there are no specific criteria for their selection.
[b]General function of the excipient in pharmaceutical preparations as mentioned in the '*Handbook of Pharmaceutical Excipients*', 6th edn.
[c]Only significant toxicities associated with the respective excipient are listed here. There may be other potential toxicities associated with the excipient. It should be noted that toxicity is dependent on the dose and route of administration. In general, these excipients are used at non-toxic dose levels in pharmaceutical formulations.
[d]ADI is acceptable daily intake as described by WHO. This information is obtained from *Hand Book of Pharmaceutical Excipients*, 6th edn.
[e]Toxicities in neonates and children.

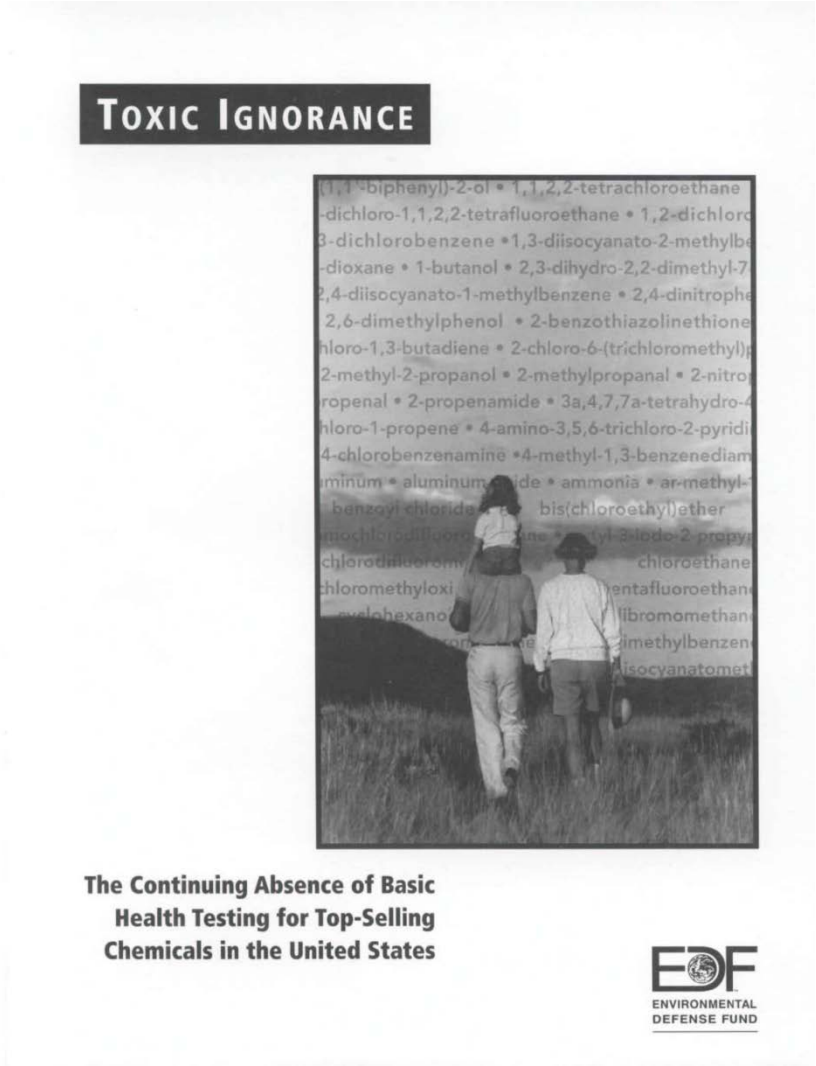
Rayavarapu et al, 2015

Chemicals

Approximately 85,000 chemicals in the US (USEPA, 2010)

A survey of High Production Volume chemicals indicates that "67% have not been tested for neurotoxicity" (EDF, 1997)

29% of chemicals have occupational exposure limits based in part, on direct neurological or behavioral effects, or on factors associated with the nervous system (Anger, 1984)



Environmental factors in Neurological Disorders

Review Article

Neuropathology and Animal Models of Autism: Genetic and Environmental Factors

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Environmental factors and multiple sclerosis

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Lancet Neurol 2008; 7: 268–77

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Studies in Canada have provided strong evidence that environmental factors act at a population level to influence the unusual geographical distribution of multiple sclerosis (MS). However, the available data accommodate more than one type of environmental effect. Migration studies show that changes to early environment can greatly affect risk, and there are recent indications that risk can be altered in situ. The rising incidence rates of MS in Canada implied by longitudinal increases in sex ratio place this effect in temporal context and narrow the candidates for mediating the effect of environment. Similarly, geographical patterns in Australia imply that modifiable environmental factors hold the key to preventing some 80% of cases. Genetic epidemiology provides overwhelming evidence that genetic background has an important complementary role. If genetic factors are held constant, the environment sets the disease threshold. Although these could be independent additive risk factors, it seems more likely that susceptibility is mediated by direct interactions between the environment and genes.

Early Environmental Origins of Neurodegenerative Disease in Later Life

Philip J. Landrigan,[1] Babasaheb Sonawane,[2] Robert N. Butler,[3] Leonardo Trasande,[1] Richard Callan,[1] and Daniel Droller[1]

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Review

Genetic and environmental factors in cancer and neurodegenerative diseases

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Received 27 February 2002; accepted in revised form 20 June 2002

Neurodegenerative Diseases: An Overview of Environmental Risk Factors

Rebecca C. Brown,[1] Alan H. Lockwood,[2] and Babasaheb R. Sonawane[3]

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The Role of Environmental Exposures in Neurodegeneration and Neurodegenerative Diseases

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Review ● Journal of INTERNAL MEDICINE

Click here for more articles from the Ceccatelli symposium

doi: 10.1111/joim.12029

Environmental stress, ageing and glial cell senescence: a novel mechanistic link to Parkinson's disease?

● S. J. Chinta*, C. A. Lieu*, M. DeMaria, R.-M. Laberge, J. Campisi & J. K. Andersen

From the Buck Institute for Research on Aging, Novato, CA, USA

Increase in critical high profile human neurological disorders in the US

~ 15 million	Depression	US $ 47 billion-anxiety (Ganz, 2006)
> 7 million	Bipolar disorder and Schizophrenia	US $ 33 billion (Ganz, 2006)
~ 5 million	Alzheimer's disease	US $ 91 billion (Ganz, 2006)
~ 5 million	Learning disabilities (primarily ADHD)	US $ 42.5 billion (Pelham et al, 2007)
2.5 million	Epilepsy	US $ 15.5 billion (Yoon et al., 2009)
1.5 million	Parkinsons ‘ disease	US $ 23 billion annually (Huse et al., 2005)
0.5 million	Autism spectrum disorders	US $ 35 billion annually (Ganz, 2006)
0.5 million	Cerebral palsy	US $ 11.5 billion (Honeycutt et al., 2004)
0.5 million	Mental retardation	US $ 51 billion (Ganz, 2006)
~ 0.5 million	Multiple Sclerosis (MS)	US $ 6.8 billion (Whetten-Goldstein et al., 1998)
0.025 million	ALS or Lou Gehrig's disease	US $ 300 million (Society for Neuroscience, 2005)

Total = ~ 38 million

Total = ~ 356.6 billion

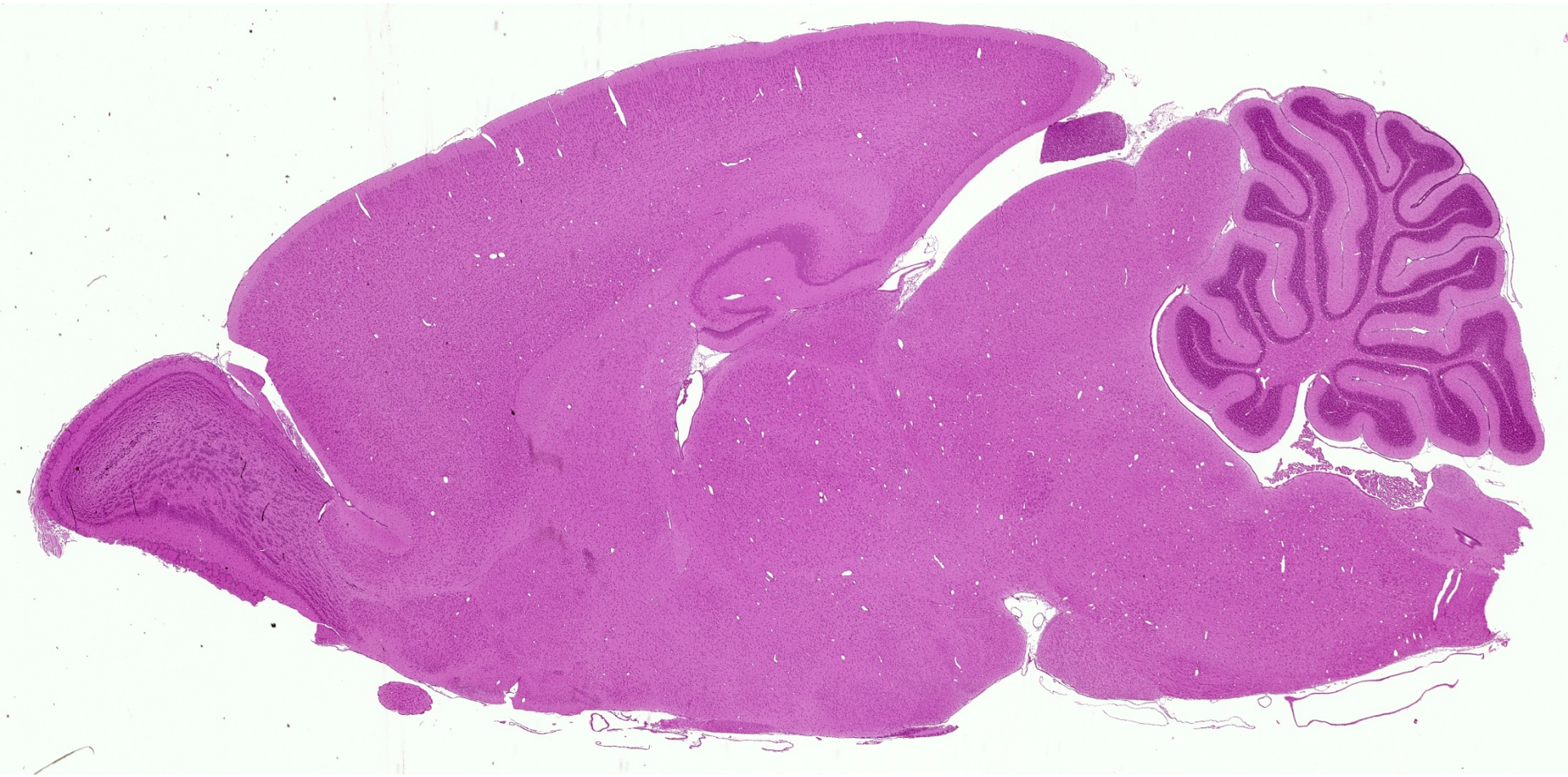
Rao, Little, Malarkey, Herbert, Sills,
Toxicologic Pathology, 39: 463, 2011

How many neurotoxicants are there ?

They exist. Drugs. Chemicals. Excipients. Unidentified.

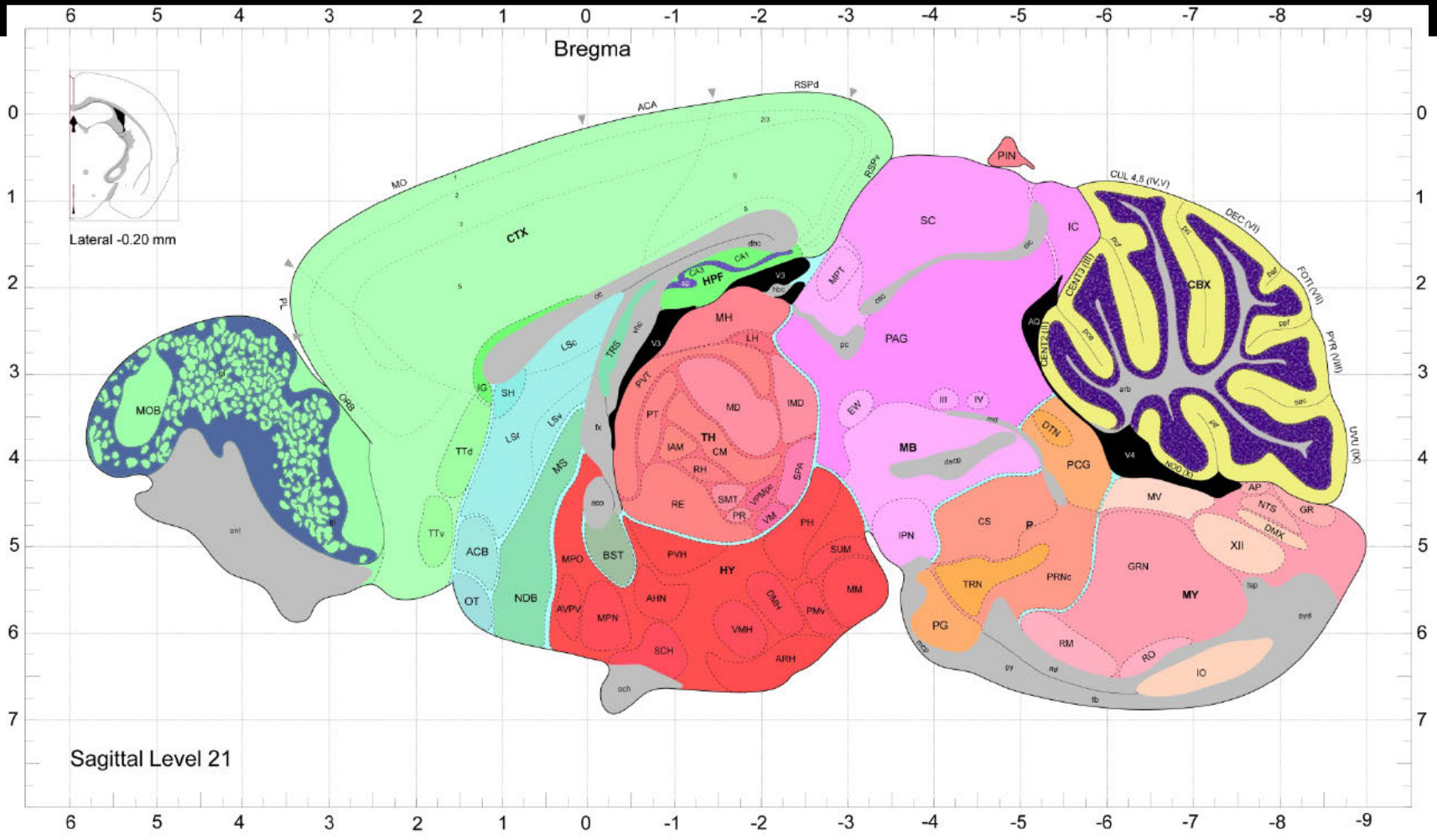
Neurotoxicity

Neurotoxicity or a neurotoxic effect is defined as an adverse change in the structure or function of the nervous system following exposure to a toxicant



The brain is remarkably heterogeneous with

> 600 distinct cell populations



Allen Institute for Brain Science

Lesion-induced deficits in Rats versus Humans

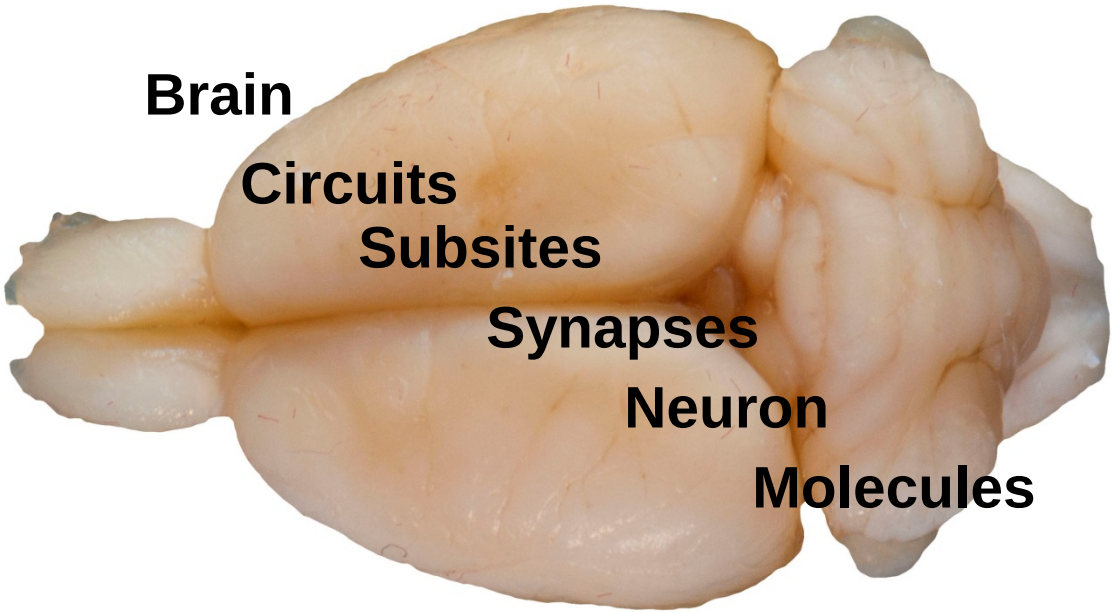
Functional Deficit	Lesion Subsite
visual discrimination	occipito-temporal cortex
maze	parietal cortex
	hippocampus
	subthalamus
	nigro-striate complex
hyperphagia	ventromedial hypothalamus
aggressiveness	septal area
	ventromedial hypothalamus
abnormality of gait	cerebellum

Lesion Subsite	Functional Deficit
occipital lobe	blindness
parietal lobe	stylus maze
hippocampus	porteus maze
subthalamus	route-walking
nigro-striate complex	hyperphagia
ventromedial hypothalamus	physical assaultiveness
septo-fornix area	rage
ventromedial hypothalamus	locomotor ataxia
cerebellum	

Thompson, R. A Behavioral Atlas of the Rat Brain

Endpoints in Neurotoxicity Assessment

Anatomic Level



End-Points

Neuropathology

Neuroimaging

Neurobehavior

(Neuro)physiology

Neurochemistry

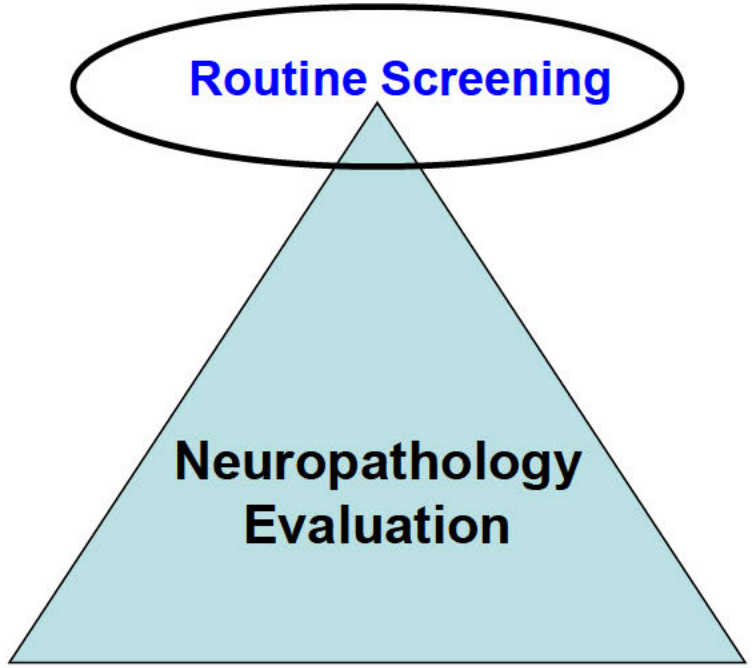
How many neurotoxicants are there ?

They exist. Drugs. Chemicals. Excipients. Unidentified.

Neurotoxicity Assessment: which endpoint to use ?

Multiple endpoints is the best approach because the nervous system is unusually complex

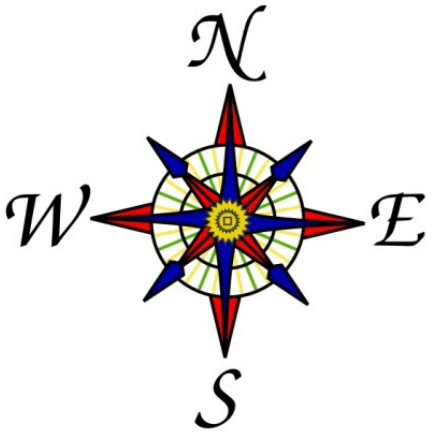
Neuropathology in Toxicology Studies



Investigative Neuropathology

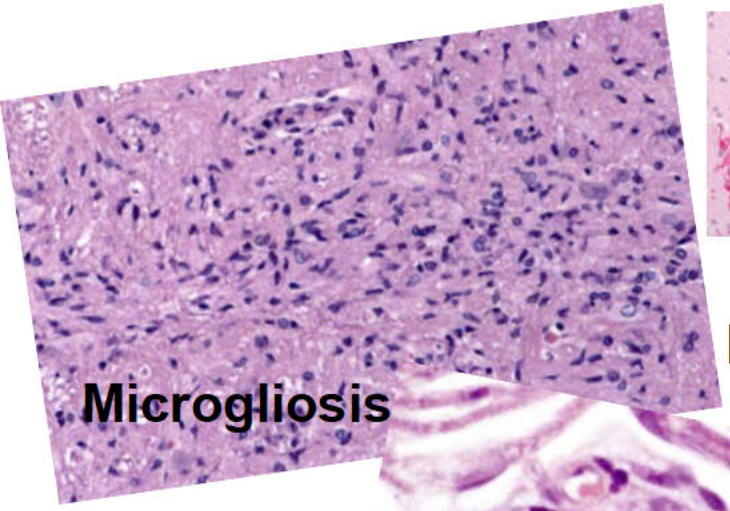
Neuropathology-Stereology, Immunohistochemistry
Neurobehavioral-Functional Observational Battery
Electrophysiology-ABER, ERG
Neuroimaging-T_2 volume
Biochemical end-points

Developmental Neuropathology

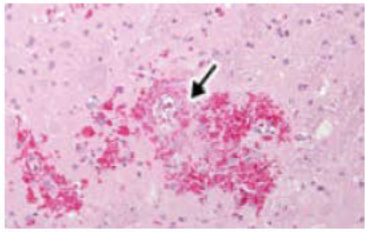


In neuropathology evaluation: Why is ‘where’ important before ‘what’?

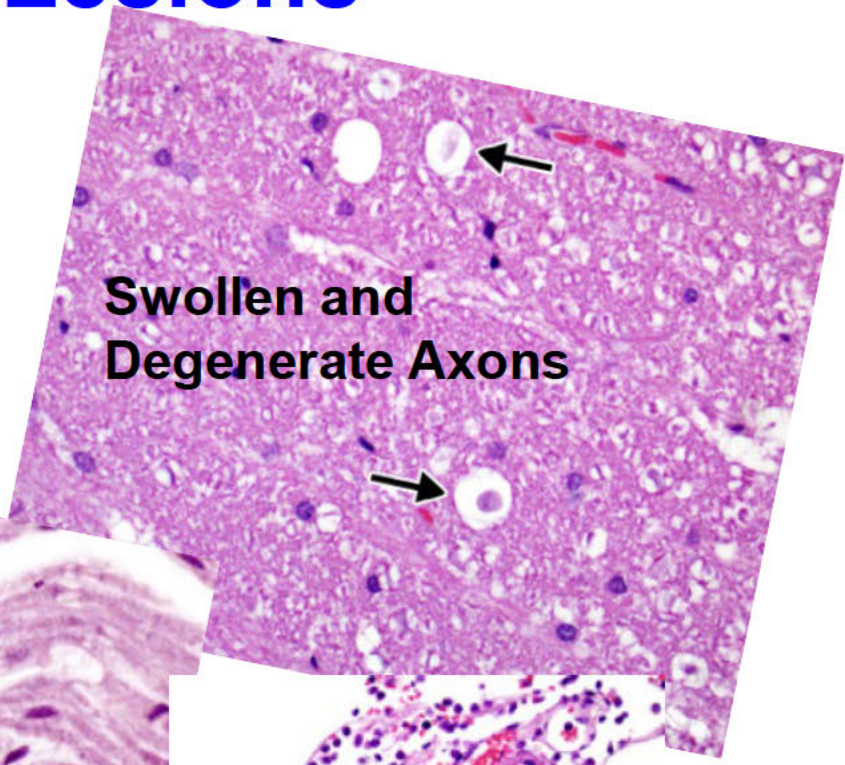
Examples of Lesions



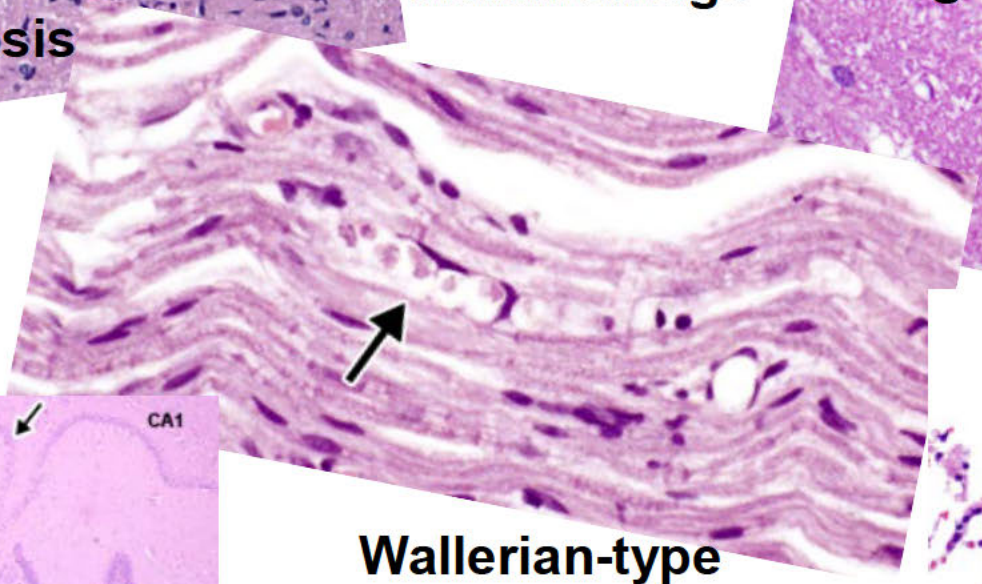
Microgliosis



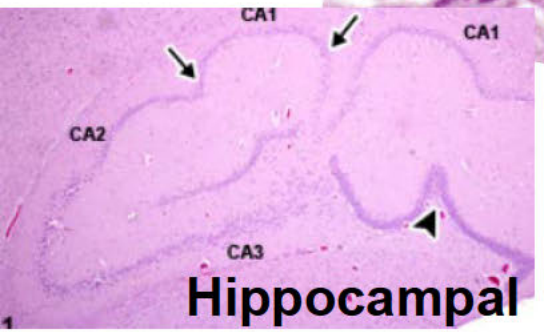
Brain Hemmorhage



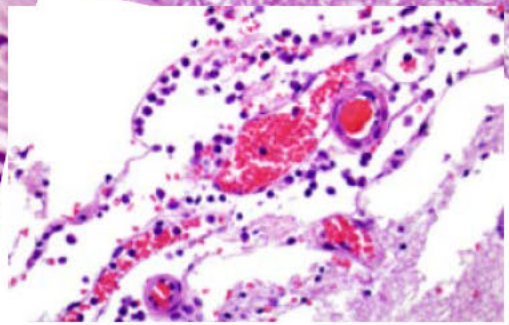
Swollen and Degenerate Axons



Wallerian-type degeneration



Hippocampal Dysplasia



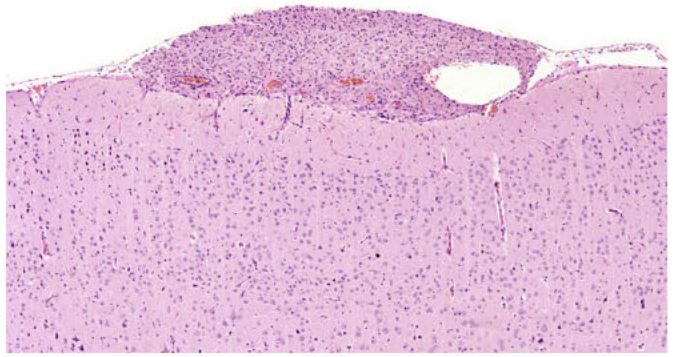
Leptomeninges inflammation

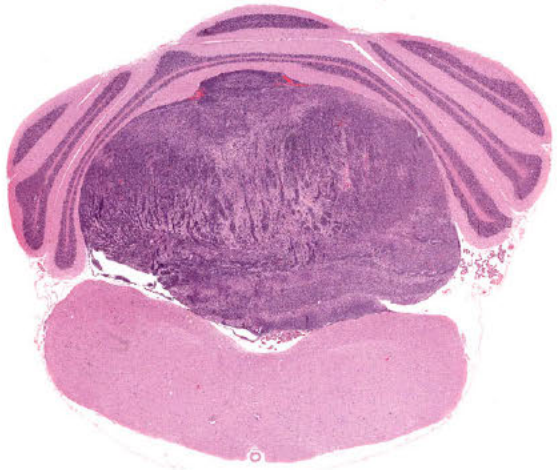
An Example with Ethylene Oxide (EO)

- Brain tumors were noted with chronic rat studies with ethylene oxide (Garman et al,1985, Garman and Snellings, 1986)

- Of 23 recorded tumors, only 2 were detected grossly. Only 3 rats demonstrated abnormal clinical signs

- Additional sectioning revealed additional tumors

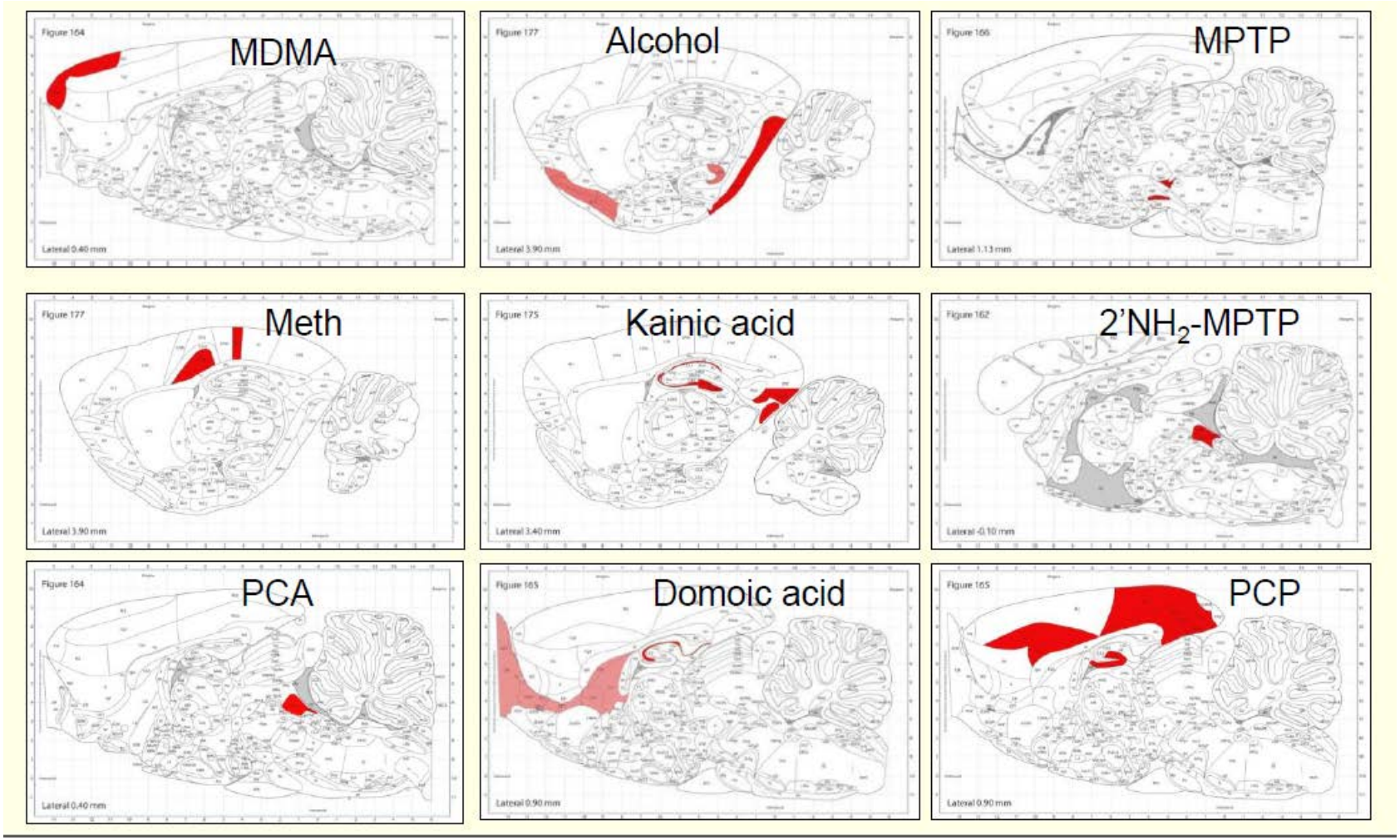




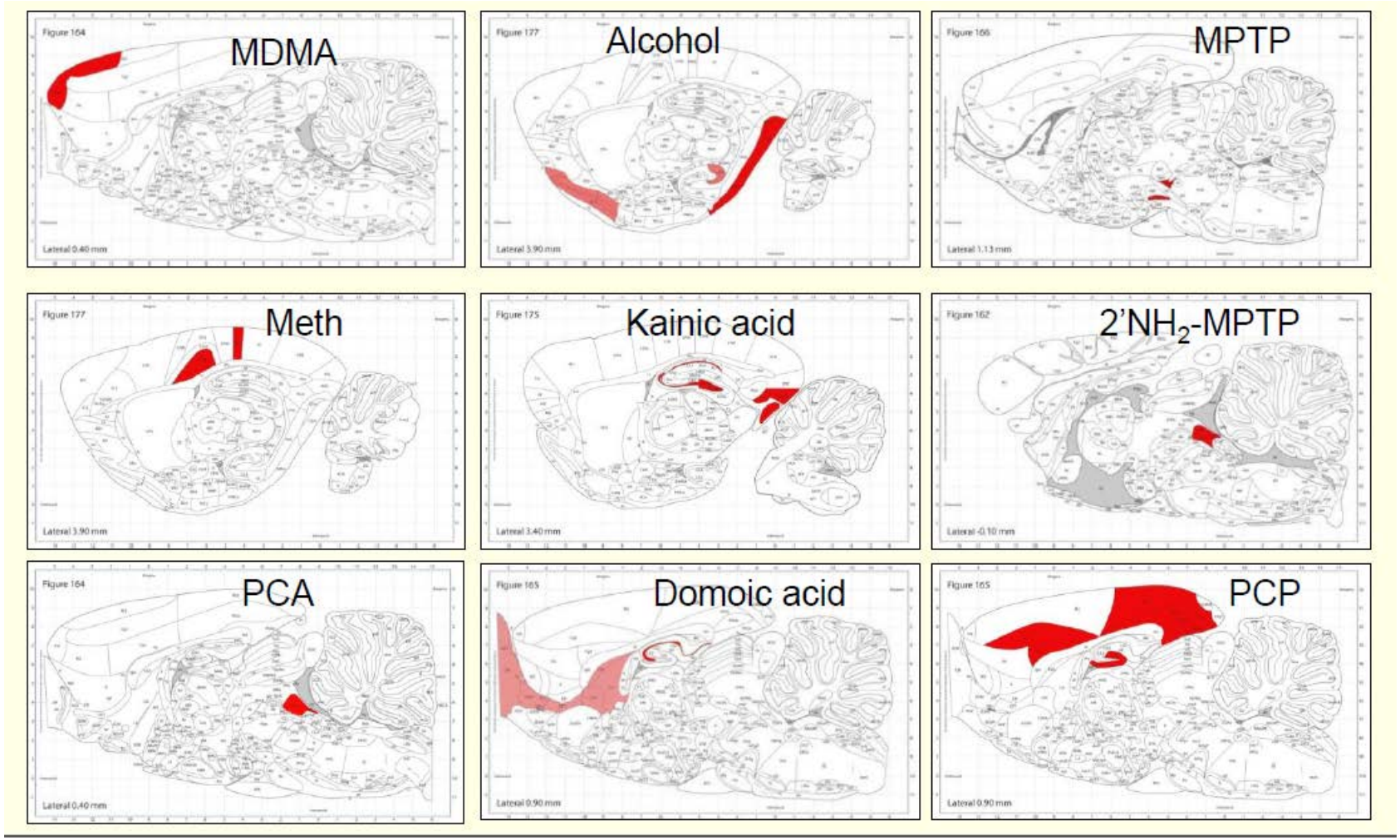
Neurotoxicants are remarkably specific

..... much depends on where we look

Neurotoxicants are remarkably specific

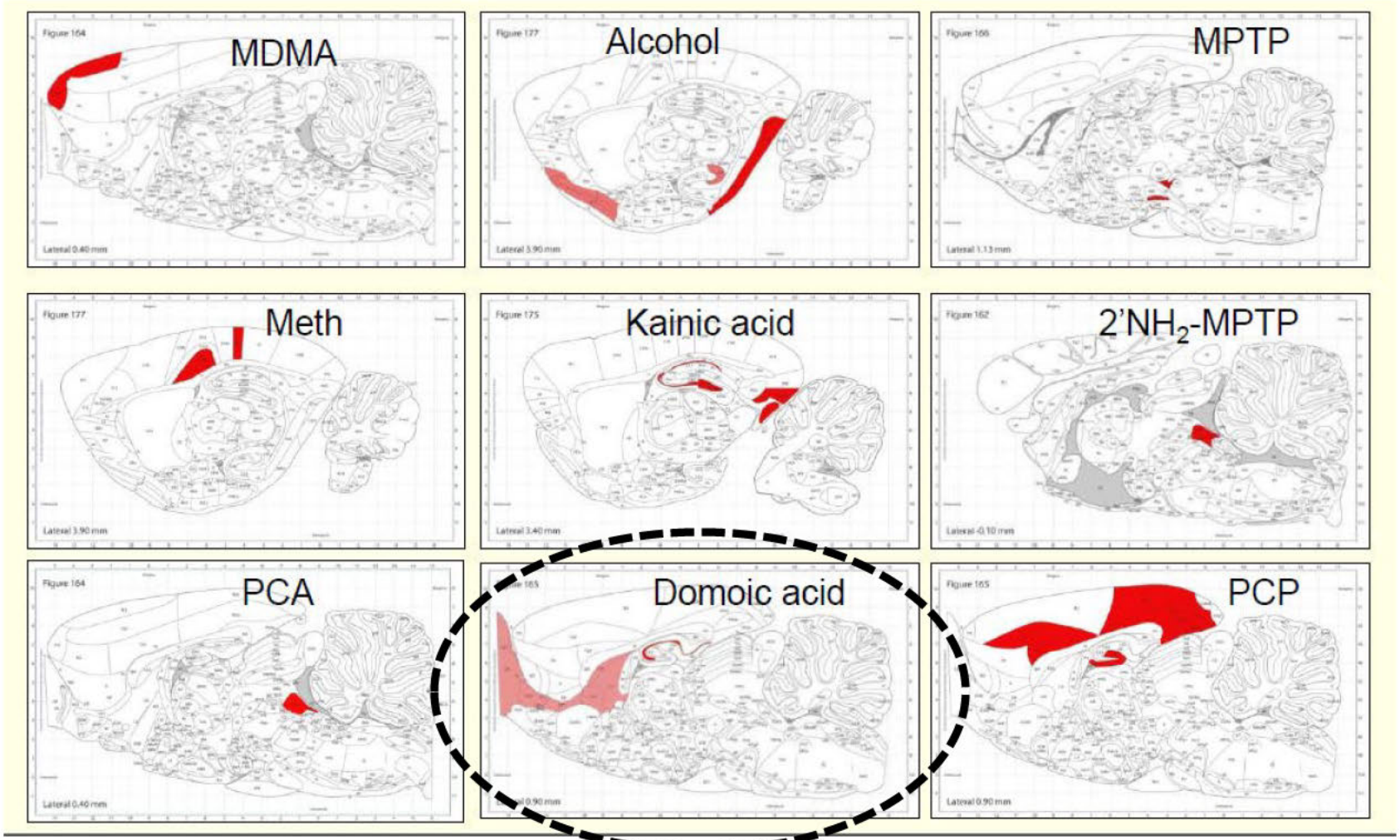


Neurotoxicants are remarkably specific



..... but much depends on where we look

Neurotoxicants are remarkably specific



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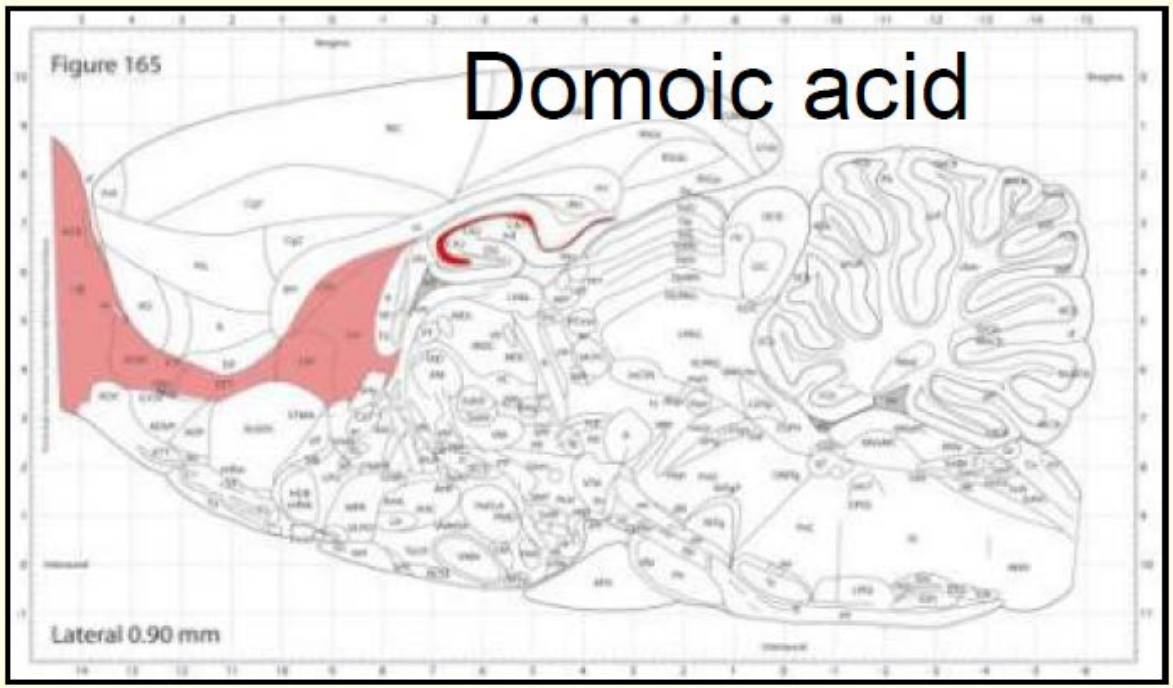
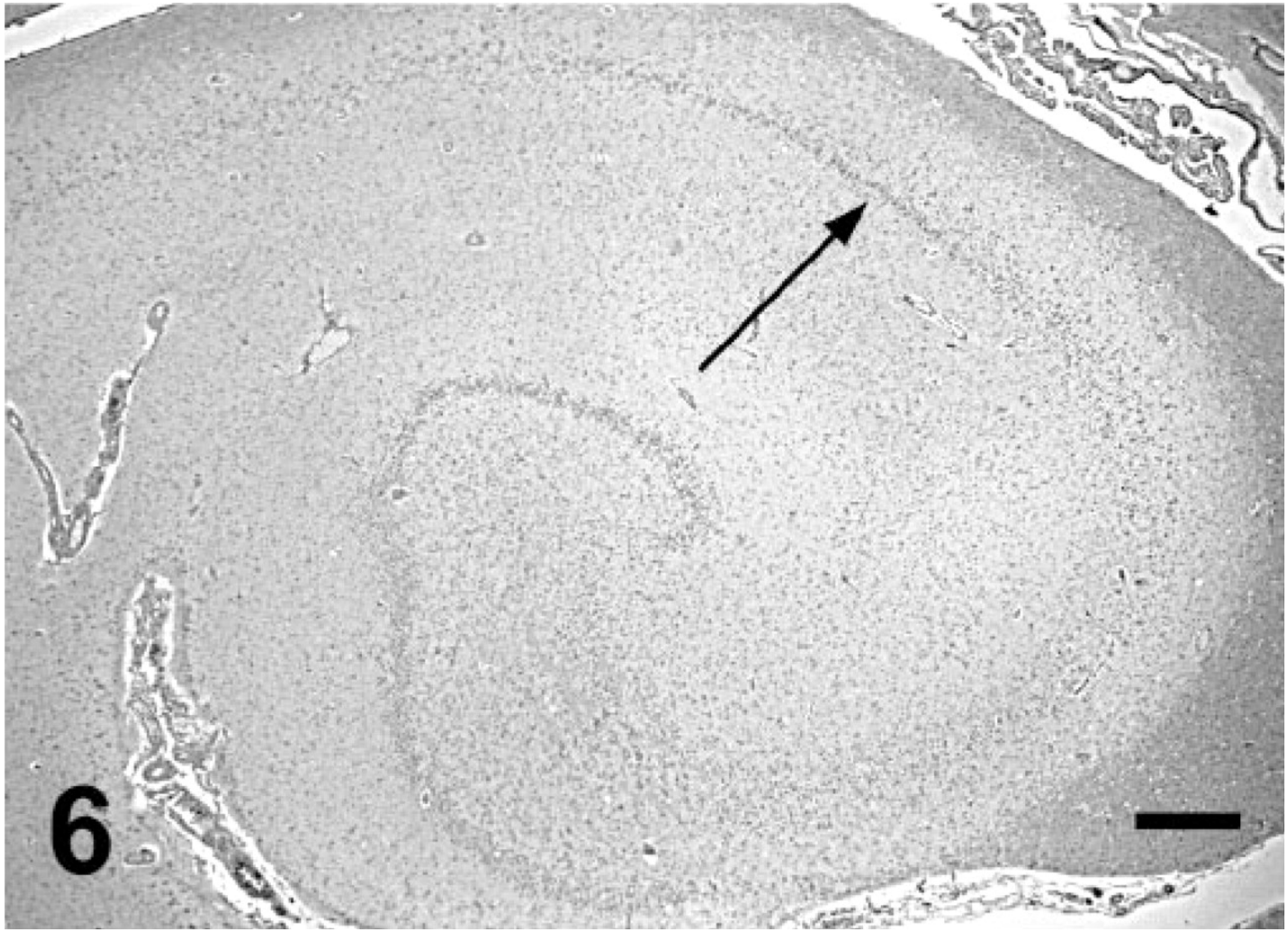


Affects the pyramidal cells of the hippocampus…..

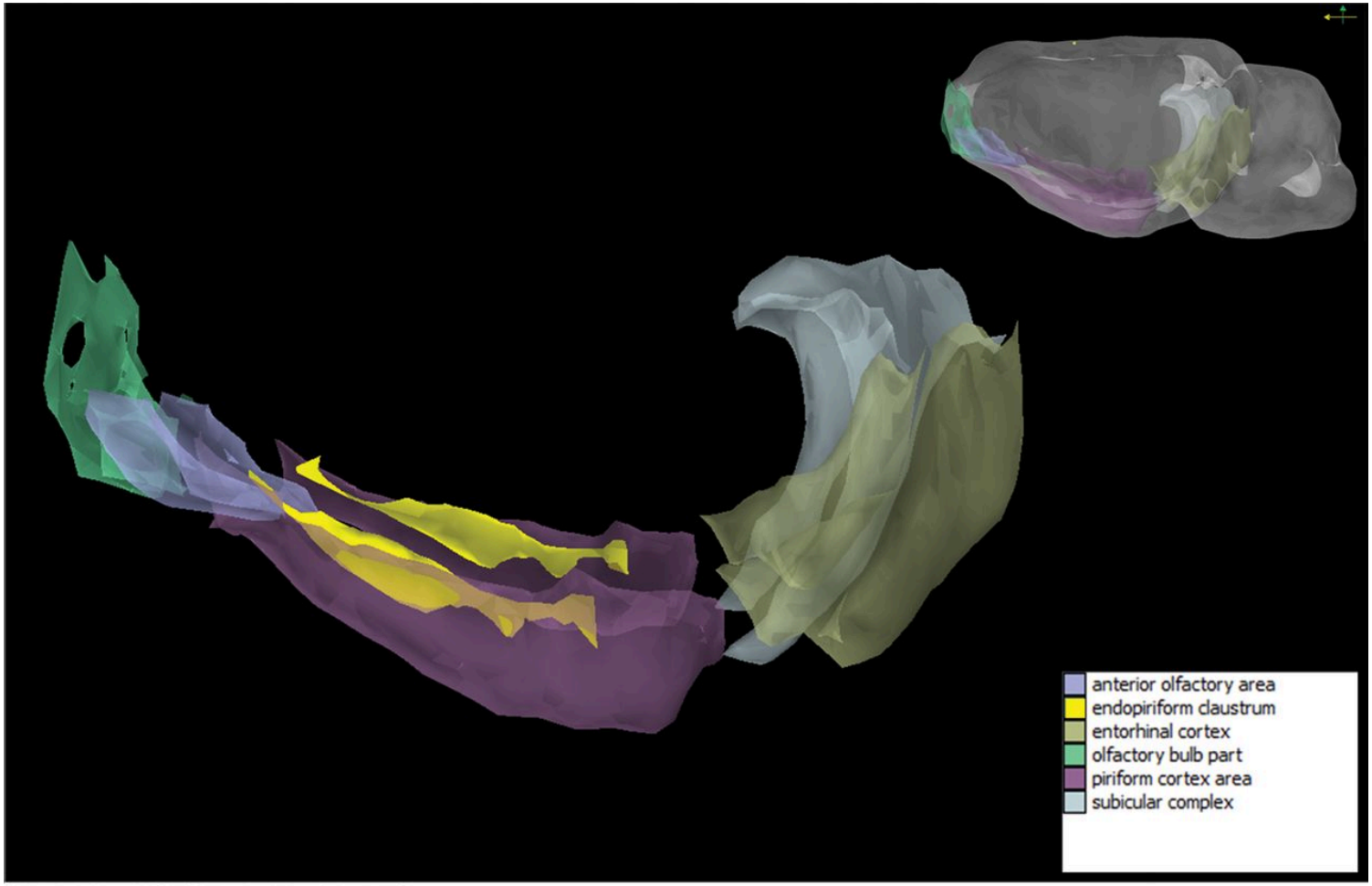
Effect of Domoic acid to the Brain: Hippocampus, California sea lion



Silvagni P A et al. Vet Pathol 2005;42:184-191

Veterinary Pathology

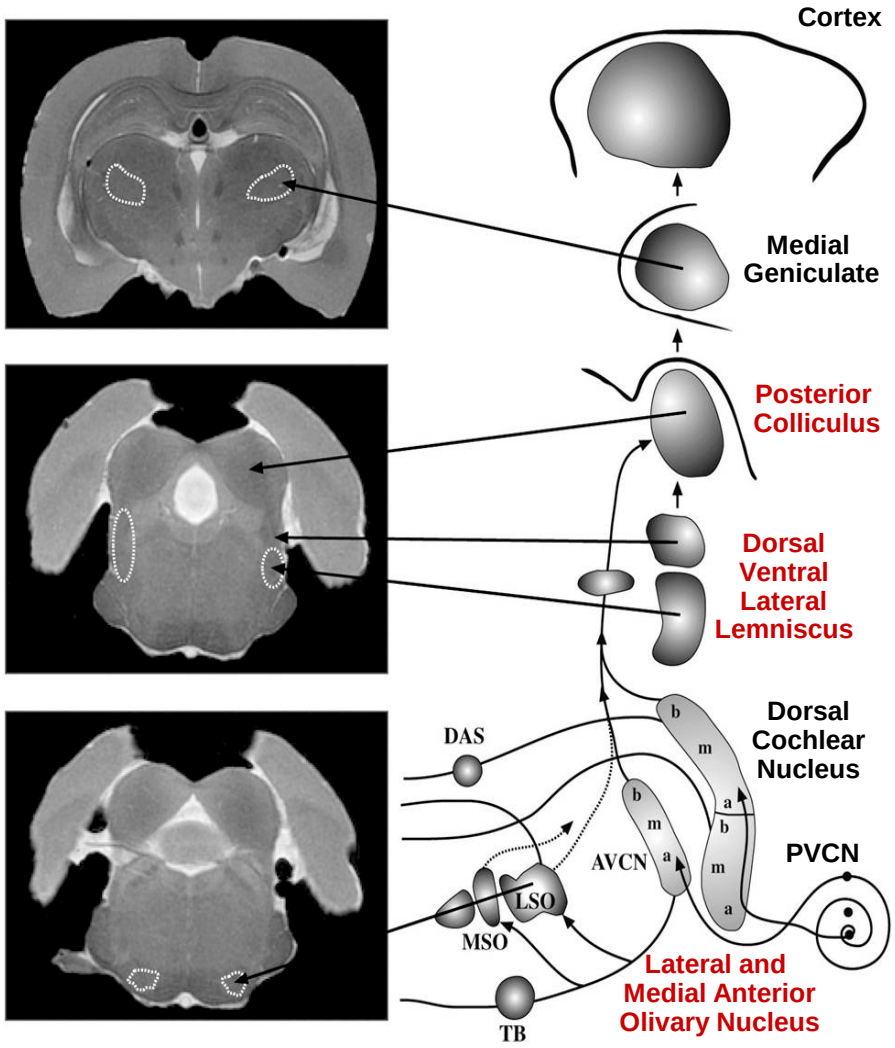
Domoic acid model depicting lesions along the olfactory pathway



Tiedeken J A et al. Toxicol Pathol 2012;41:454-469

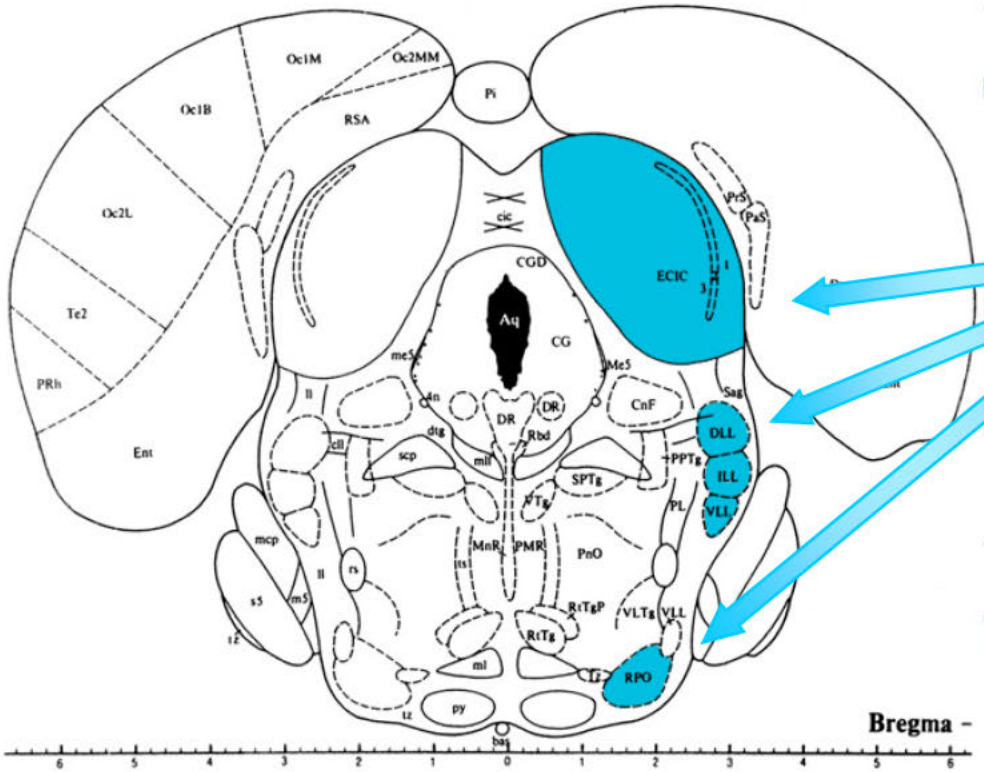
TOXICOLOGIC PATHOLOGY

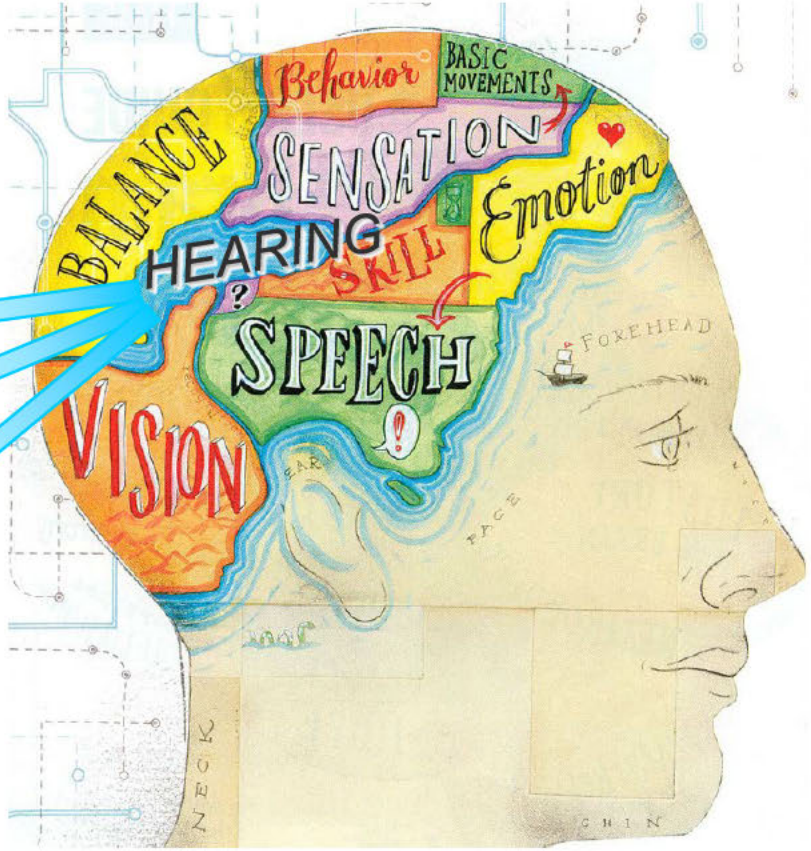
Carbonyl Sulfide - Auditory Pathway



Sills, Morgan, Herr, Little, George, Ton, Love, Maronpot, Johnson, Toxicologic Pathology, 32: 501, 2004

The New Map Of The Brain: Link Sites ➡ Function





Times, January 29, 2007

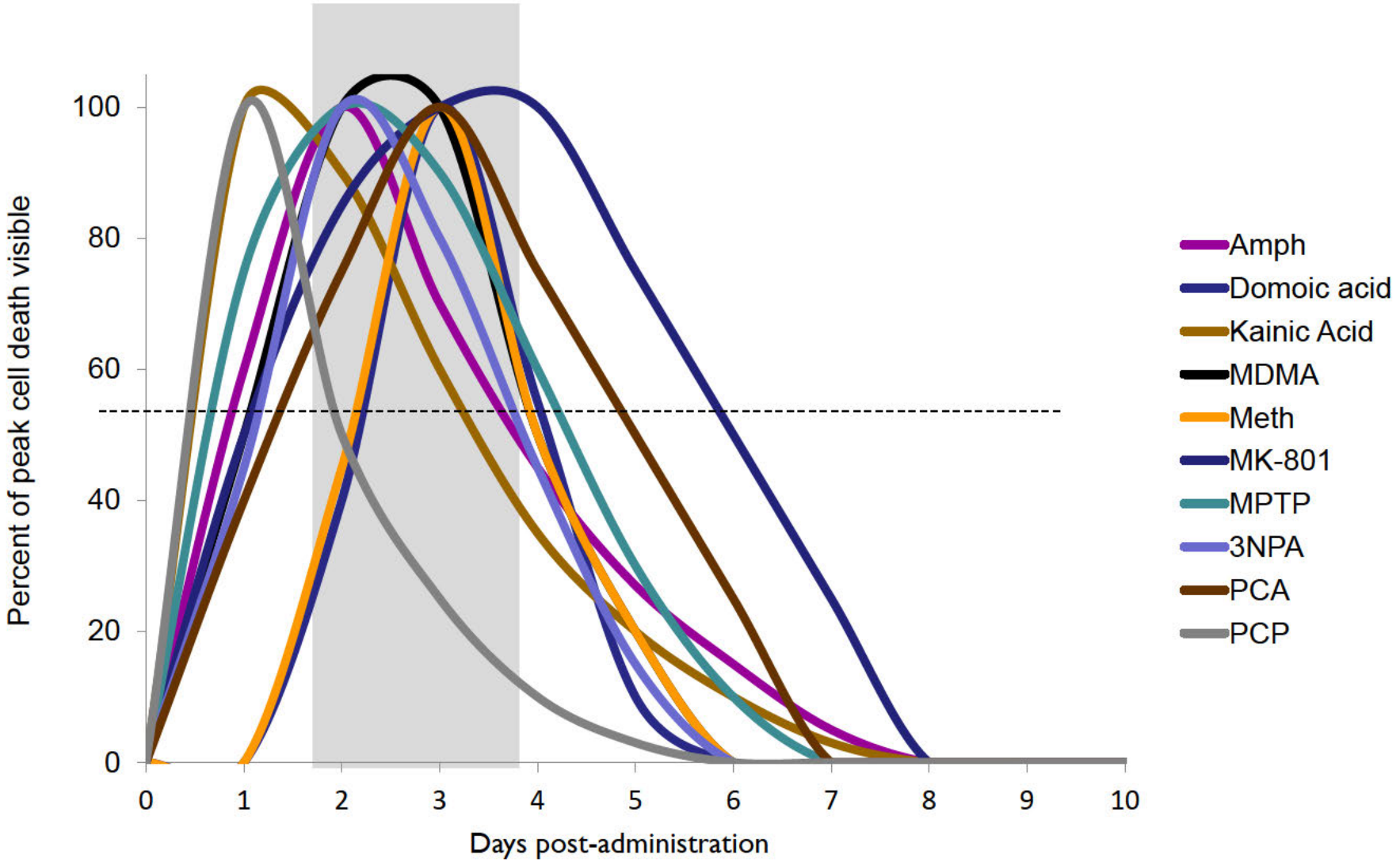
How many neurotoxicants are there ?
They exist. Drugs. Chemicals. Excipients. Unidentified.

Neurotoxicity Assessment:
which endpoint to use ?
Multiple endpoints is the best approach because
the nervous system is unusually complex

Key Factors for Neuropathology

1) Location – is it part of a neuroanatomical circuit ?

In addition to location: Timing is important



From: RC Switzer III; NeuroScience Associates

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Position Paper

Toxicologic Pathology, 39: 463-470, 2011

ISSN: 0192-6233 print / 1533-1601 online
DOI: 10.1177/0192623311401044

Histopathological Evaluation of the Nervous System in National Toxicology Program Rodent Studies: A Modified Approach

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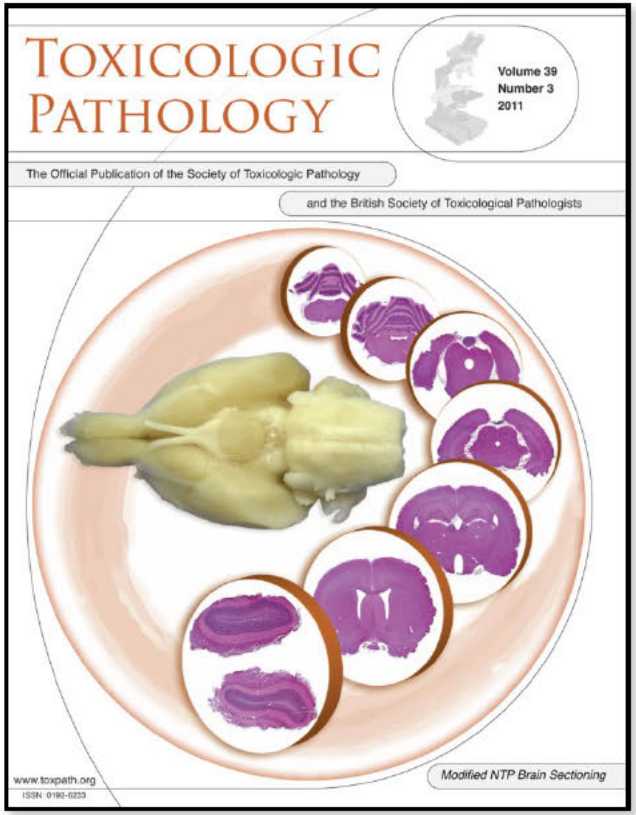
[2]*Integrated Laboratory Systems, Inc., Research Triangle Park, North Carolina, USA*
[3]*Charles River Laboratories, Pathology Associates, Durham, North Carolina, USA*

ABSTRACT

This article outlines the changes and underlying rationale for modifications to the histopathological evaluation of the nervous system during toxicology and carcinogenesis studies conducted by the National Toxicology Program (NTP). In the past, routine evaluation of the nervous system was mostly limited to three sections of brain, and occasionally the spinal cord and peripheral nerves. Factors such as the increasing occurrence of human neurological diseases and associated economical cost burden, the role of unidentified environmental stressors in neurodegenerative disorders, multiple therapeutic drug-induced neuropathies noted in human clinical trials, and the exponential use of environmental chemicals with unknown neurotoxic potential necessitate a more extensive evaluation of the nervous system. The NTP has modified its protocol to include examination of key anatomic subsites related to neurodegenerative diseases such as Parkinson's disease. Modifications include four additional sections of the brain. Increasing the number of brain sections permits examination of a greater number of specific anatomic subsites with unique vulnerability. In addition, the spinal cord, peripheral nerves, trigeminal ganglion, and intestinal autonomic ganglia will be evaluated as needed. It is expected that this modified approach will increase the sensitivity of detecting neurotoxicants and neurocarcinogens important in human neurologic and neurodegenerative disorders.

Keywords: neuropathology; histopathology; brain; nervous system; NTP; nervous system; screening.



Rao, Little, Malarkey, Herbert, Sills, Toxicologic Pathology, 39: 463, 2011

j. **Seven** cross-sections of the brain **taken at levels (brain matrix molds may be used) shown in Figure 5a (rat) and 5b (mouse)** shall include: (1) **olfactory bulb (mid-level); (2) fronto-parietal** cortex **including** basal ganglia **(1-2 mm cranial to the optic chiasma)**; **(3) mid-**parietal cortex and thalamus **(mid-point of the infundibulum); (4) mid-brain with substantia nigra and red nucleus (mid-point of anterior colliculus); (5) posterior colliculi (mid-point of posterior calliculus); (6) mid-cerebellum including cranial nerve VIII; and (7) posterior medulla through the area postrema (2-3 mm anterior to termination of the cerebellum). If small brains preclude obtaining seven quality sections, a minimum of five sections shall be obtained to include sections 1-4 and 6 corresponding to Figure 5b below. Sections 'b' through 'h' shall be placed in the cassette (large cassette for thick sections) with rostral surface down for sectioning.** If any lesions are observed after sectioning, they shall be noted on the IANR.

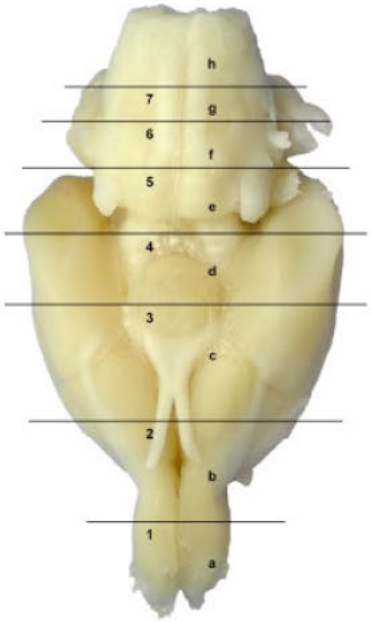


Figure 5a

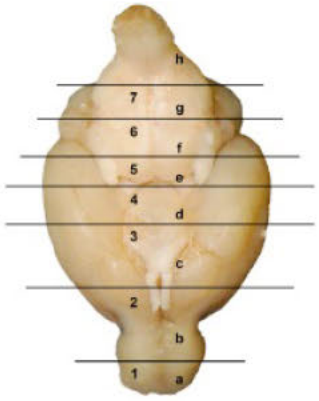
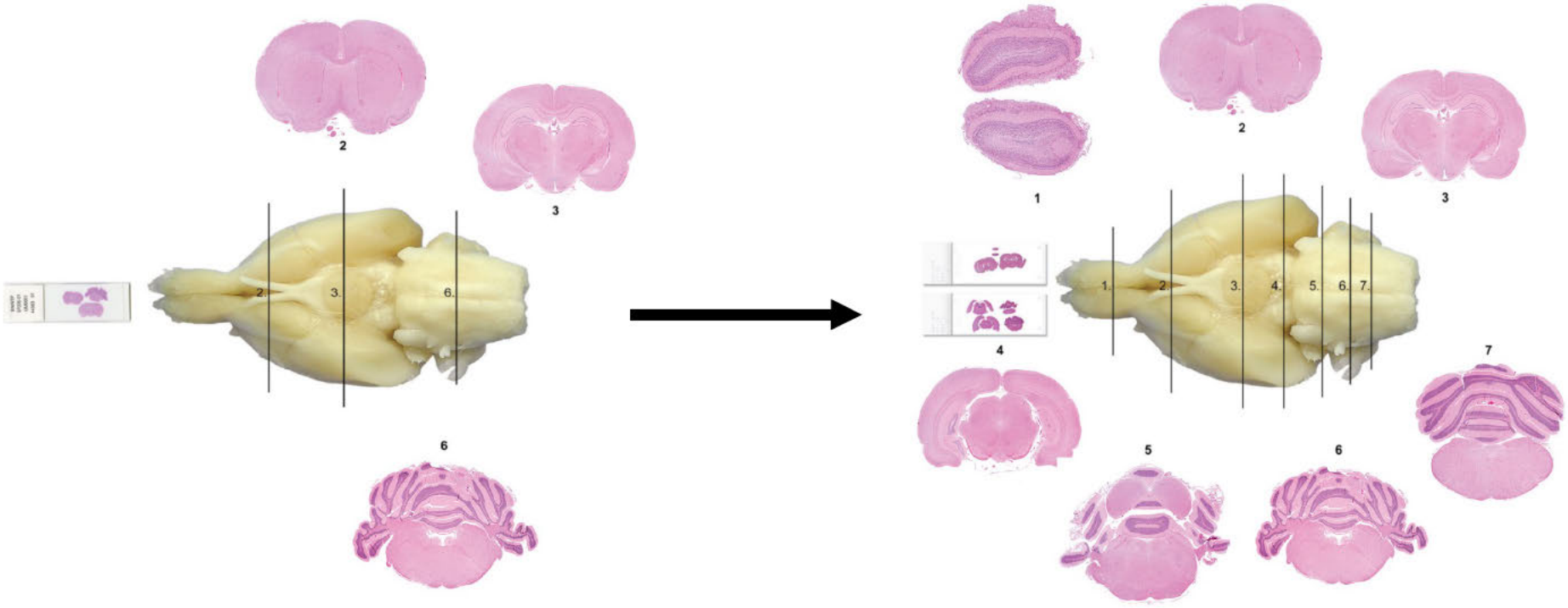


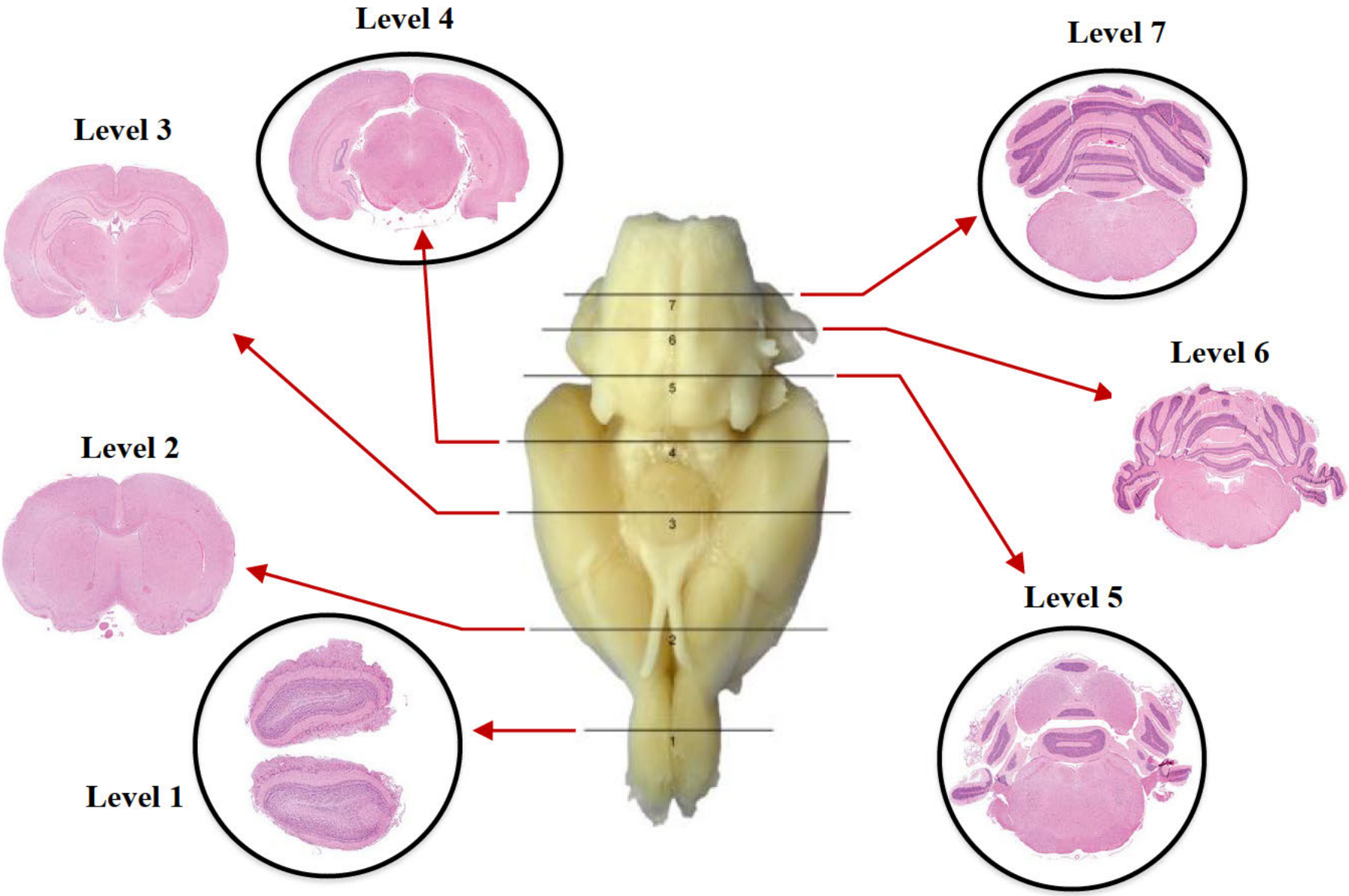
Figure 5b

B6C3F1 Mouse

SPECIFICATIONS
FOR THE CONDUCT OF STUDIES
TO EVALUATE THE TOXIC AND CARCINOGENIC POTENTIAL
OF CHEMICAL, BIOLOGICAL AND PHYSICAL AGENTS
IN LABORATORY ANIMALS
FOR THE NATIONAL TOXICOLOGY PROGRAM (NTP)

Three sections to Seven Sections




Level 4
Level 7
Level 3
Level 6
Level 2
Level 5
Level 1
7
6
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4
3
2
1

Scientific and Regulatory Policy Paper

Toxicologic Pathology, 41: 1028-1048, 2013

ISSN: 0192-6233 print / 1533-1601 online
DOI: 10.1177/0192623312474865

STP Position Paper: Recommended Practices for Sampling and Processing the Nervous System (Brain, Spinal Cord, Nerve, and Eye) during Nonclinical General Toxicity Studies

Brad Bolon[1], Robert H. Garman[2], Ingrid D. Pardo[3], Karl Jensen[4], Robert C. Sills[5], Aude Roulois[6], Ann Radovsky[7], Alys Bradley[8], Lydia Andrews-Jones[9], Mark Butt[10], and Laura Gumprecht[11]

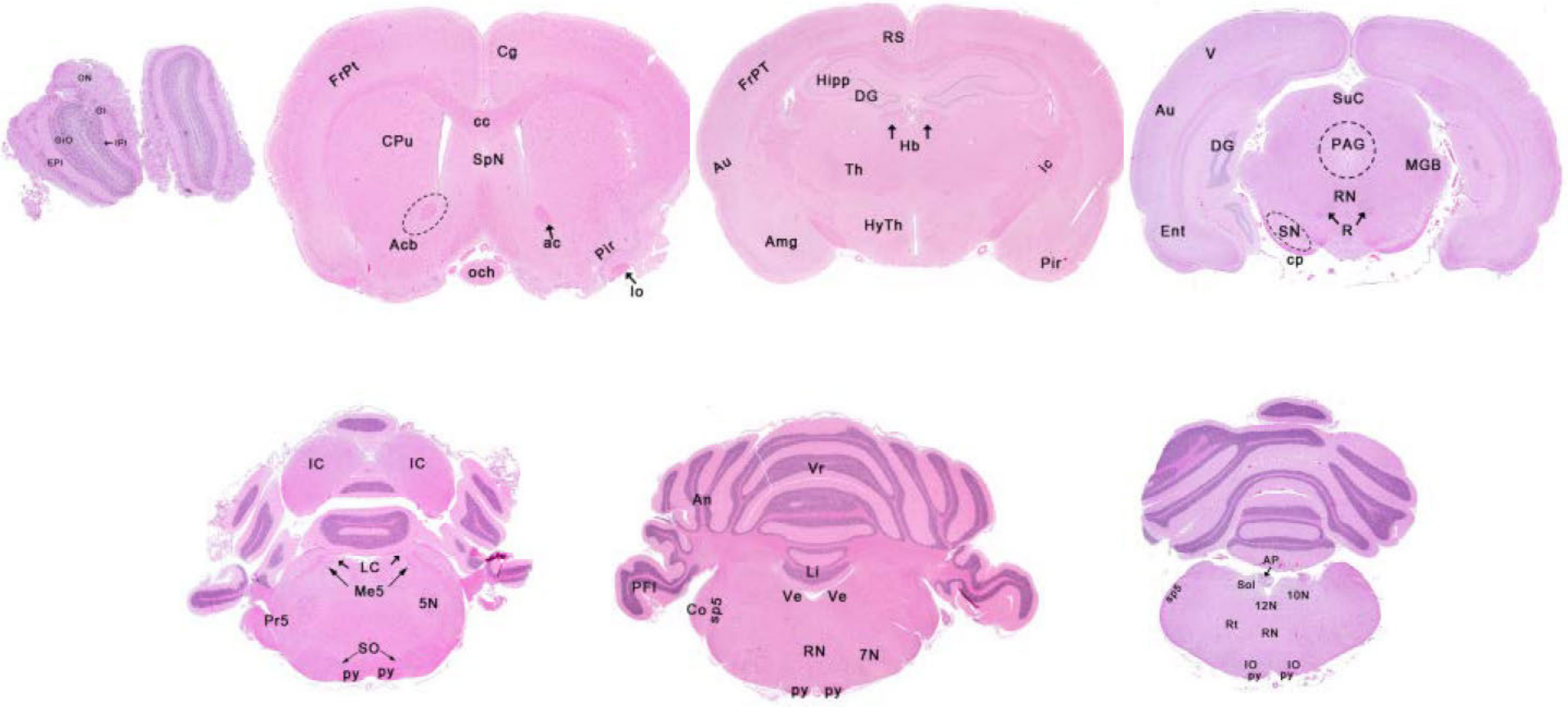
[1]*The Ohio State University, Columbus, Ohio, USA*
[2]*Consultants in Veterinary Pathology, Inc., Murrysville, Pennsylvania, USA*
[3]*Pfizer, Inc., Groton, Connecticut, USA*
[4]*U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA*
[5]*U.S. National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA*
[6]*GlaxoSmithKline, Ware, United Kingdom*
[7]*WIL Research, Ashland, Ohio, USA*
[8]*Charles River Laboratories, Edinburgh, Scotland*
[9]*Allergan, Irvine, California, USA*
[10]*Tox Path Specialists, LLC, Frederick, Maryland, USA*
[11]*Merck Research Laboratories, West Point, Pennsylvania, USA*

Abstract

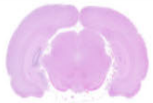
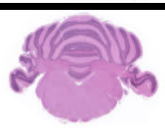
The Society of Toxicologic Pathology charged a Nervous System Sampling Working Group with devising recommended practices to routinely screen the central nervous system (CNS) and peripheral nervous system (PNS) in Good Laboratory Practice–type nonclinical general toxicity studies. Brains should be weighed and trimmed similarly for all animals in a study. Certain structures should be sampled regularly: caudate/putamen, cerebellum, cerebral cortex, choroid plexus, eye (with optic nerve), hippocampus, hypothalamus, medulla oblongata, midbrain, nerve, olfactory bulb (rodents only), pons, spinal cord, and thalamus. Brain regions may be sampled bilaterally in rodents using 6 to 7 coronal sections, and unilaterally in nonrodents with 6 to 7 coronal hemisections. Spinal cord and nerves should be examined in transverse and longitudinal (or oblique) orientations. Most Working Group members considered immersion fixation in formalin (for CNS or PNS) or a solution containing acetic acid (for eye), paraffin embedding, and initial evaluation limited to hematoxylin and eosin (H&E)-stained sections to be acceptable for routine microscopic evaluation during general toxicity studies; other neurohistological methods may be undertaken if needed to better characterize H&E findings. Initial microscopic analyses should be qualitative and done with foreknowledge of treatments and doses (i.e., "unblinded"). The pathology report should clearly communicate structures that were assessed and methodological details. Since neuropathologic assessment is only one aspect of general toxicity studies, institutions should retain flexibility in customizing their sampling, processing, analytical, and reporting procedures as long as major neural targets are evaluated systematically.

Keywords: brain; brain weight; CNS; eye; general toxicity study; GLP; nervous system; neuropathology; neurotoxicity; nonclinical toxicity study; PNS; recommended practices; spinal cord.

Neuroanatomic areas in the NTP-7 brain sectioning protocol



Neuroanatomic subsites

NEUROANATOMIC SUBSITES		
	**NTP-7 Level 1**	olfactory bulb (olfactory nerve layer, glomerular layer, external and internal plexiform layers, mitral cell layer, and granule cell layer)
	**NTP-7 Level 2**	fronto-parietal cortex, cingulate cortex, corpus callosum, caudate-putamen, (internal capsule, globus pallidus), septal nuclei, anterior commissure, accumbens nucleus, piriform cortex, optic chiasm or nerve, lateral olfactory tract
	**NTP-7 Level 3**	fronto-parietal cortex, retrosplenial cortex, auditory (temporal) cortex, amygdaloid nuclei, hippocampus (CA regions - 1,2,3, dentate gyrus), habenular nucleus, thalamus, hypothalamus, internal capsule, (globus pallidus)
	**NTP-7 Level 4**	visual (occipital) cortex, auditory (temporal) cortex, entorhinal cortex, superior colliculus, periaqueductal gray, medial geniculate body, red nucleus, raphe nuclei, cerebral peduncle, substantia nigra
	**NTP-7 Level 5**	inferior colliculus, locus coeruleus, mesencephalic trigeminal nucleus, principal sensory nucleus of CN V, motor trigeminal nucleus, superior olivary nucleus, pyramidal tracts
	**NTP-7 Level 6**	cerebellar lobules (vermis, ansiform, paraflocculus, lingula), facial (CN VII) nucleus, spinal trigeminal tract, vestibular nucleus, cochlear nucleus, raphe nuclei, pyramidal tracts
	**NTP-7 Level 7**	area postrema, solitary tract nucleus, vagal (CV X) nucleus, hypoglossal (CN XII) nucleus, reticular formation, raphe nuclei, inferior olivary nucleus, pyramidal tracts, spinal trigeminal tract

NeuroToxicology 42 (2014) 12–23

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NeuroToxicology

Quantitative mapping of trimethyltin injury in the rat brain using magnetic resonance histology

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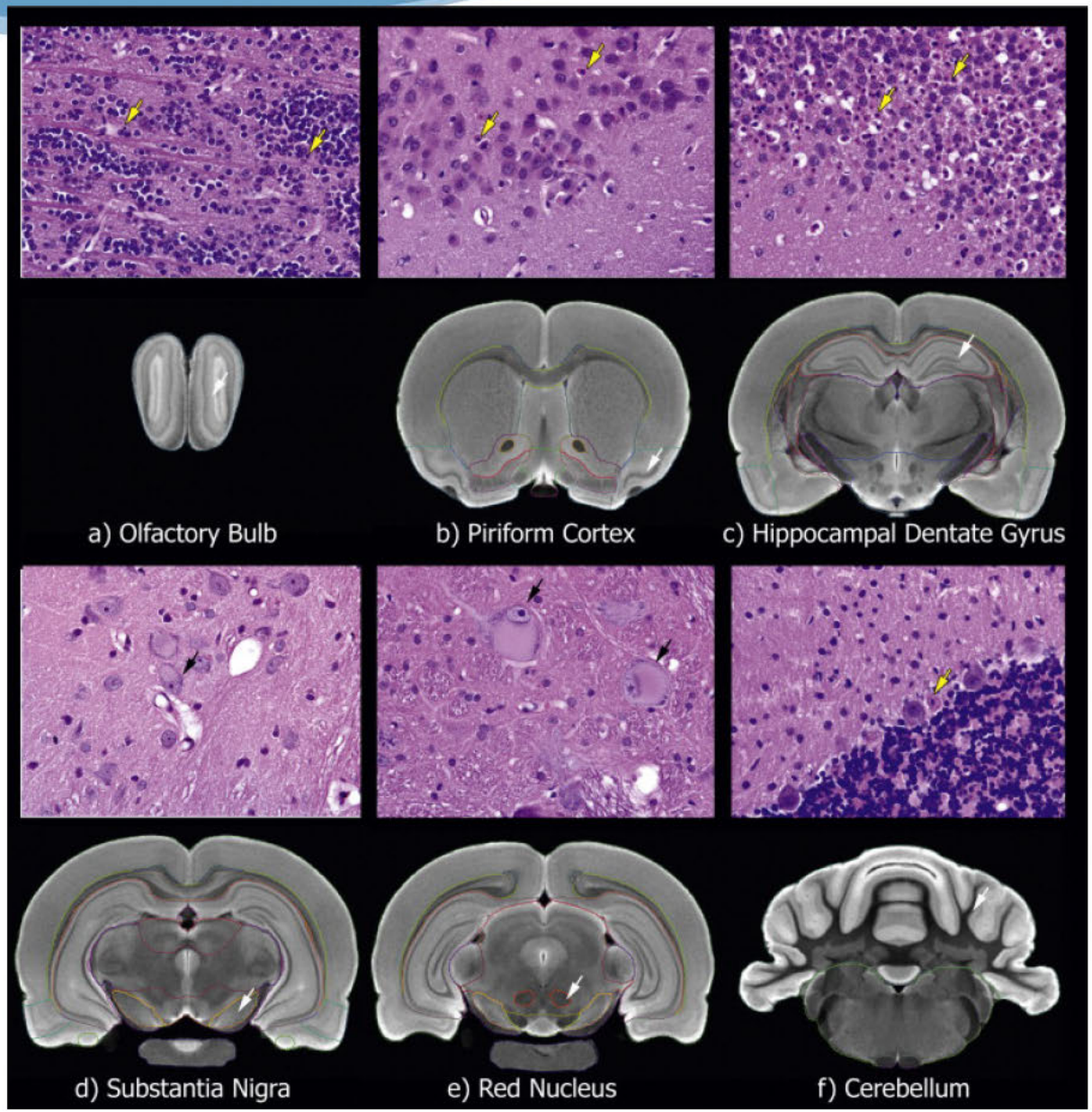
ABSTRACT

The growing exposure to chemicals in our environment and the increasing concern over their impact on health have elevated the need for new methods for surveying the detrimental effects of these compounds. Today's gold standard for assessing the effects of toxicants on the brain is based on hematoxylin and eosin (H&E)-stained histology, sometimes accompanied by special stains or immunohistochemistry for neural processes and myelin. This approach is time-consuming and is usually limited to a fraction of the total brain volume. We demonstrate that magnetic resonance histology (MRH) can be used for quantitatively assessing the effects of central nervous system toxicants in rat models. We show that subtle and sparse changes to brain structure can be detected using magnetic resonance histology, and correspond to some of the locations in which lesions are found by traditional pathological examination. We report for the first time diffusion tensor image-based detection of changes in white matter regions, including fimbria and corpus callosum, in the brains of rats exposed to 8 mg/kg and 12 mg/kg trimethyltin. Besides detecting brain-wide changes, magnetic resonance histology provides a quantitative assessment of dose-dependent effects. These effects can be found in different magnetic resonance contrast mechanisms, providing multivariate biomarkers for the same spatial location. In this study, deformation-based morphometry detected areas where previous studies have detected cell loss, while voxel-wise analyses of diffusion tensor parameters revealed microstructural changes due to such things as cellular swelling, apoptosis, and inflammation. Magnetic resonance histology brings a valuable addition to pathology with the ability to generate brain-wide quantitative parametric maps for markers of toxic insults in the rodent brain.



Johnson et al 2014 Neurotoxicology 42: 12-23

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Multiple endpoints is the best approach because the nervous system is unusually complex

Key Factors for Neuropathology

1) Location – is it part of a neuroanatomical circuit ?
2) Timing

Neuropathology Evaluation:
how many sections ?
Routine – Seven sections *at least*
Should be hypothesis driven
based on what we know about the molecule