DNTP Neuropathology Study Set

Division of the National Toxicology Program

Comparative and Molecular Pathogenesis Branch

National Institute of Environmental Health Sciences (NIEHS)

DNTP Neuropathology Study Set Details

This study set uses DNTP Archival study material of compounds known to have neurological clinical signs and neuropathological effects.

Note to trainees and others using this study set

- This study set consists of scanned slides derived from 10 neurotoxic compounds.
- The set was compiled from study and review of Central and Peripheral nervous system (CNS and PNS) slides, many of which consisted of the then-NTP standard 3 cross sections of brain. Those sections were the frontal cortical section including basal ganglia (now NTP-7 level 2), infundibulum including thalamus and hippocampal structures (now NTP-7 level 3), and a mid medullary/cerebellar section (now NTP-7 level 6). See Slide 6 for these sections in the NTP-7 overview.
- The new NTP brain trim protocol now uses seven (7) sections plus spinal cord, sciatic and tibial nerves. See the illustration showing levels 1-7 on Slide 5.
- Persons using this set are advised to first examine closely the narrative and images included in the central nervous system (CNS) section of the Non-neoplastic Lesion Atlas, which can be accessed here: https://ntp.niehs.nih.gov/nnl/nervous/index.htm

Note to trainees and others using this study set

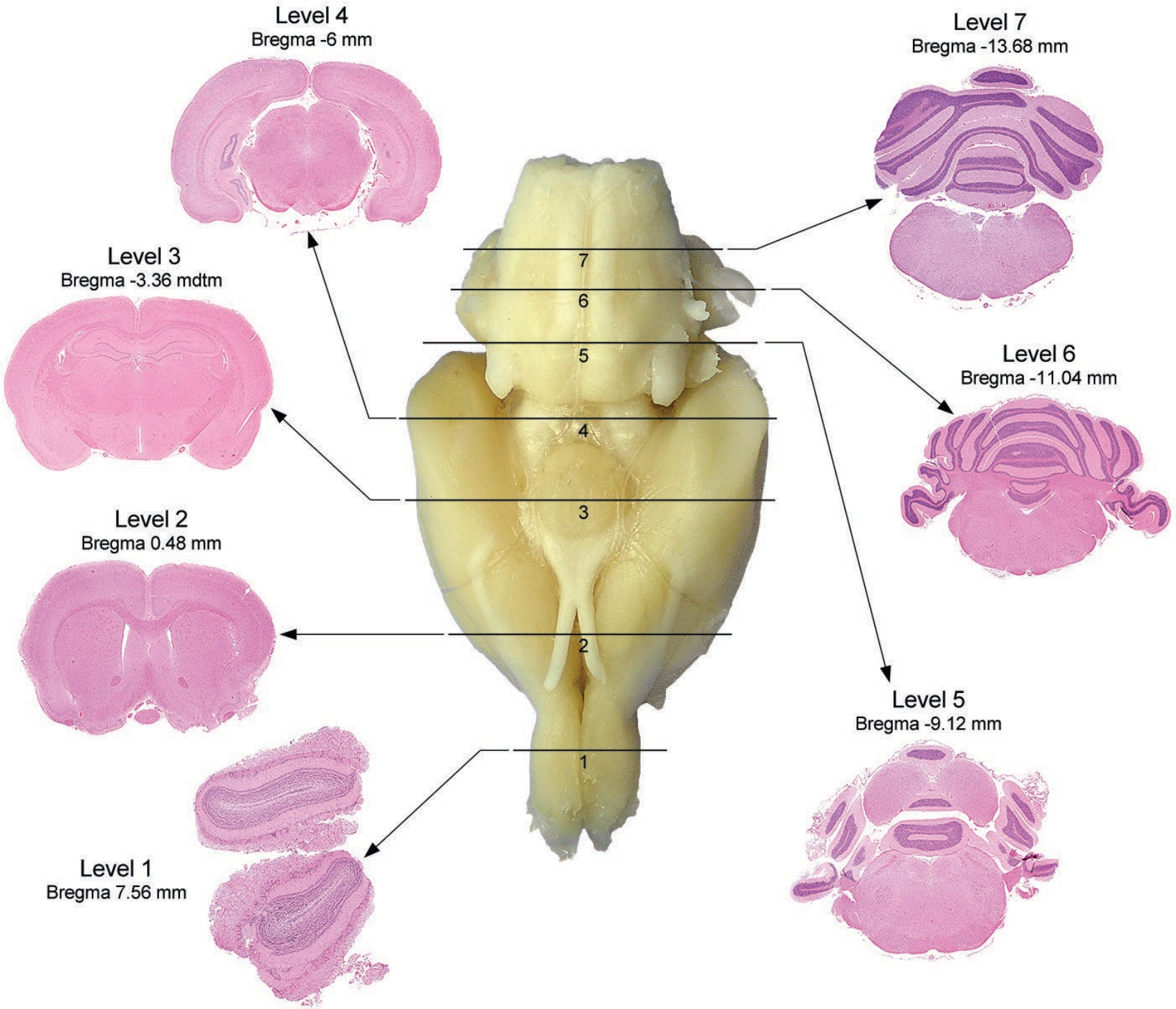
- The study set and presentation are intended to be used during and after review of the whole slide images on-line with Concentriq, the NTP/NIEHS Image Management System. Much of the neuropathological material and lesions depicted include often subtle features requiring close examination and study.
- Annotations and high-power images have been included in the whole slide images to assist the user in recognizing these features. Instructions for accessing these annotations is provided in “Instructions for using Concentriq”.
- It is expected that the user will examine the PowerPoint slides that provide compound and clinical information followed by examination of the scanned slides blindly and finally the PowerPoint slides which provide details of neuropathologic lesions and the list of approved diagnoses.

Instructions for using Concentriq

- Access the NIEHS Image Management System (Concentriq) here: https://niehsimages.epl-inc.com/imageSets/114
  - This whole slide image set is publicly available and does not require you to log in to the system.
- Click on the whole slide image name in the left panel to view the scan.
- You can navigate the whole slide image using your mouse by clicking and dragging. The image is responsive to the mouse scroll wheel to zoom in and out. You can also use the magnification slider bar in the lower left to zoom in and out.
- To view annotations and related whole slide image metadata, click on the notepad icon in the lower right corner.

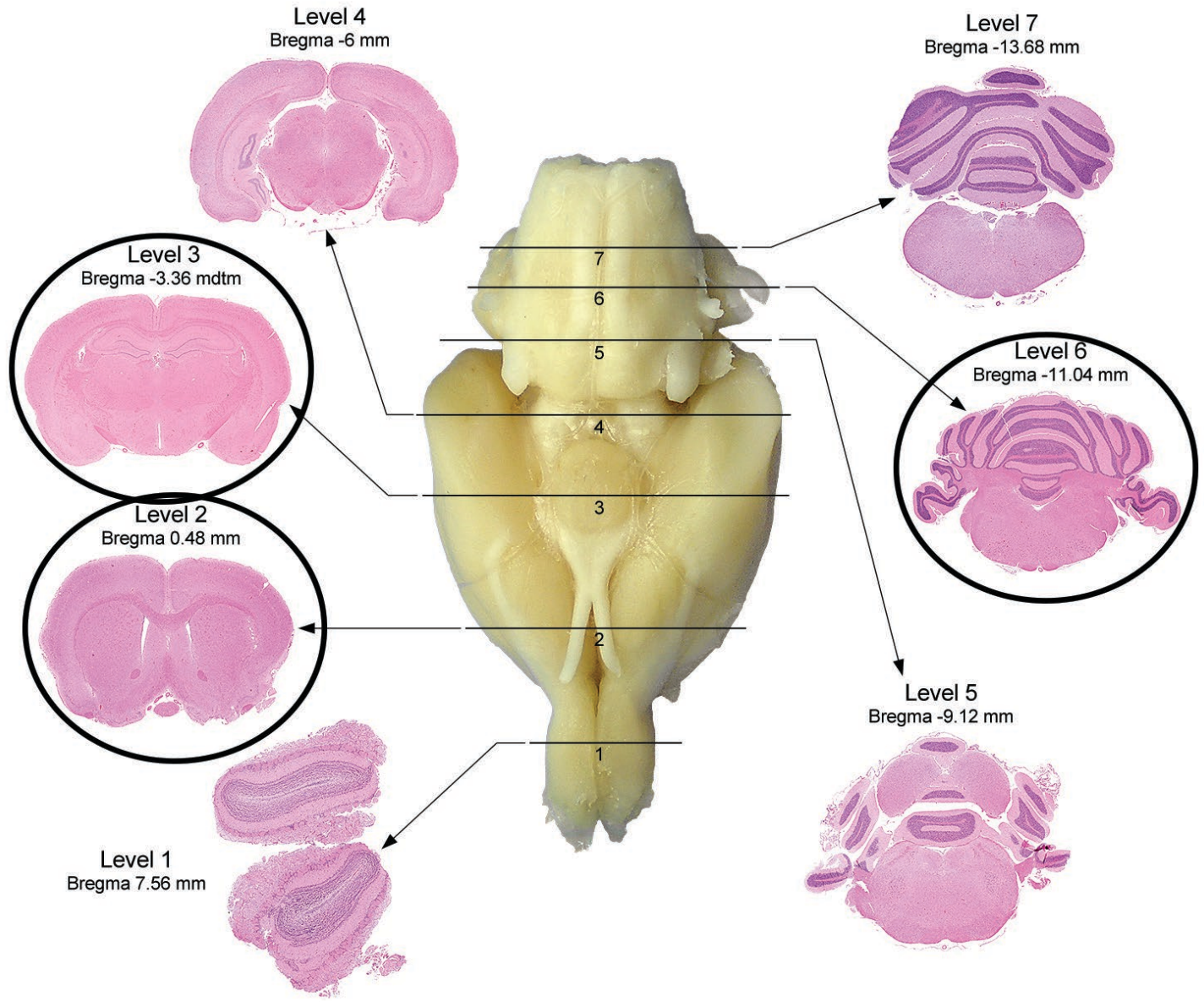
National Institute of Environmental Health Sciences
Division of the National Toxicology Program

NTP-7 Overview



NTP-7 Overview

- Levels 2, 3, and 6 correspond to the previous NTP 3-section method



Learn more about the NTP-7

See the following seven (7) slides for detail on the neuroanatomy of each site in the NTP-7 brain sections.

Further detail can be obtained by accessing Paxinos, G., & Watson, C. (2013). The rat brain in stereotaxic coordinates (7th ed.) [E-book]. Elsevier Gezondheidszorg. eBook ISBN 9780124157521

See also:
Rao, D. B., Little, P. B., Malarkey, D. E., Herbert, R. A., & Sills, R. C. (2011). Histopathological evaluation of the nervous system in national toxicology program rodent studies. Toxicologic Pathology, 39(3), 463–470. https://doi.org/10.1177/0192623311401044

Rao, D. B., Little, P. B., & Sills, R. C. (2013). Subsite awareness in neuropathology evaluation of national toxicology program (NTP) studies. Toxicologic Pathology, 42(3), 487–509. https://doi.org/10.1177/0192623313501893

NTP-7 Level 1

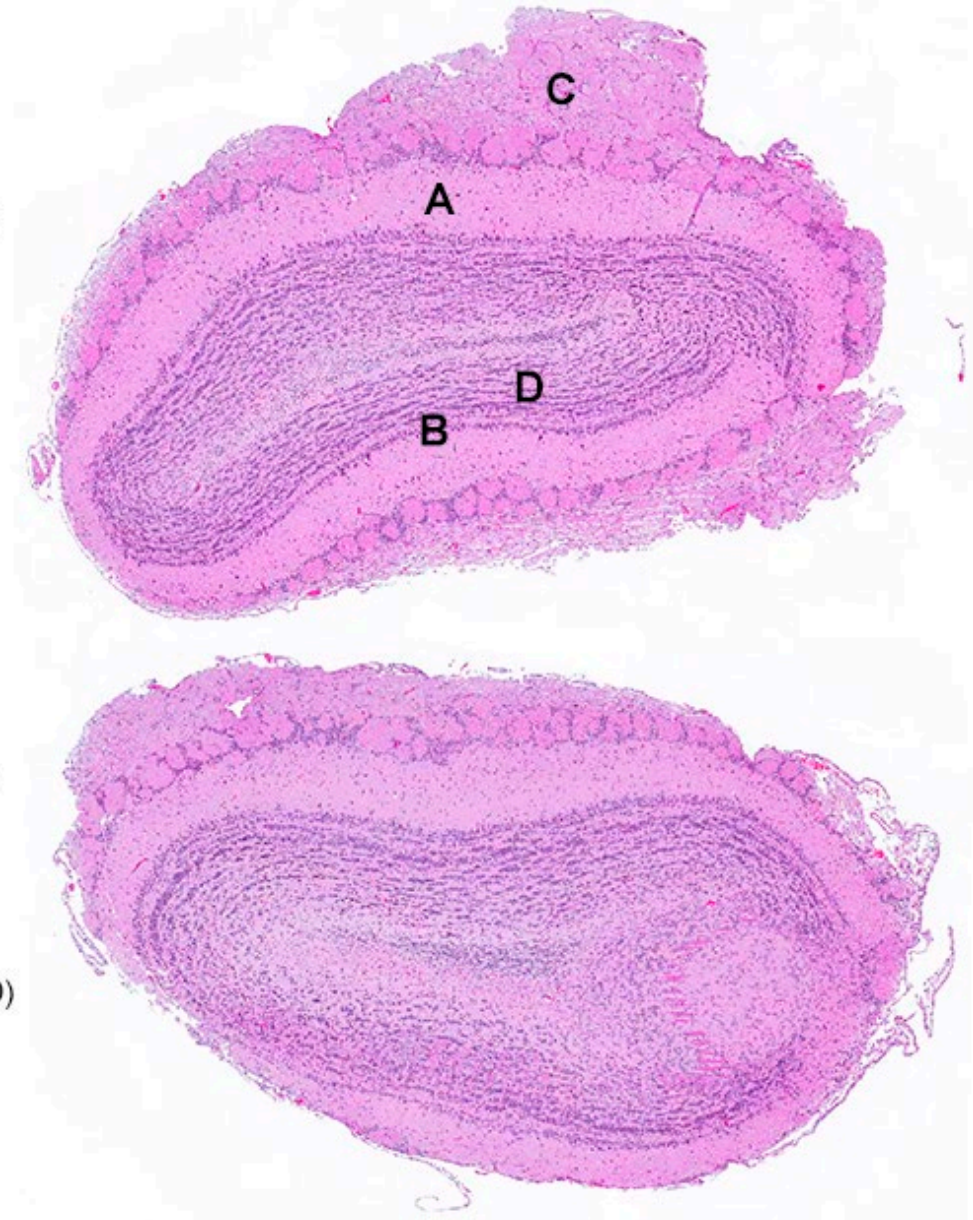
Olfactory Bulb:

Plexiform Layer (A)

Mitral Layer (B)

Glomerular Layer (C)

Granule Cell Layer (D)



NTP-7 Level 2

Parietal Cortex (A)

Piriform Cortex (B)

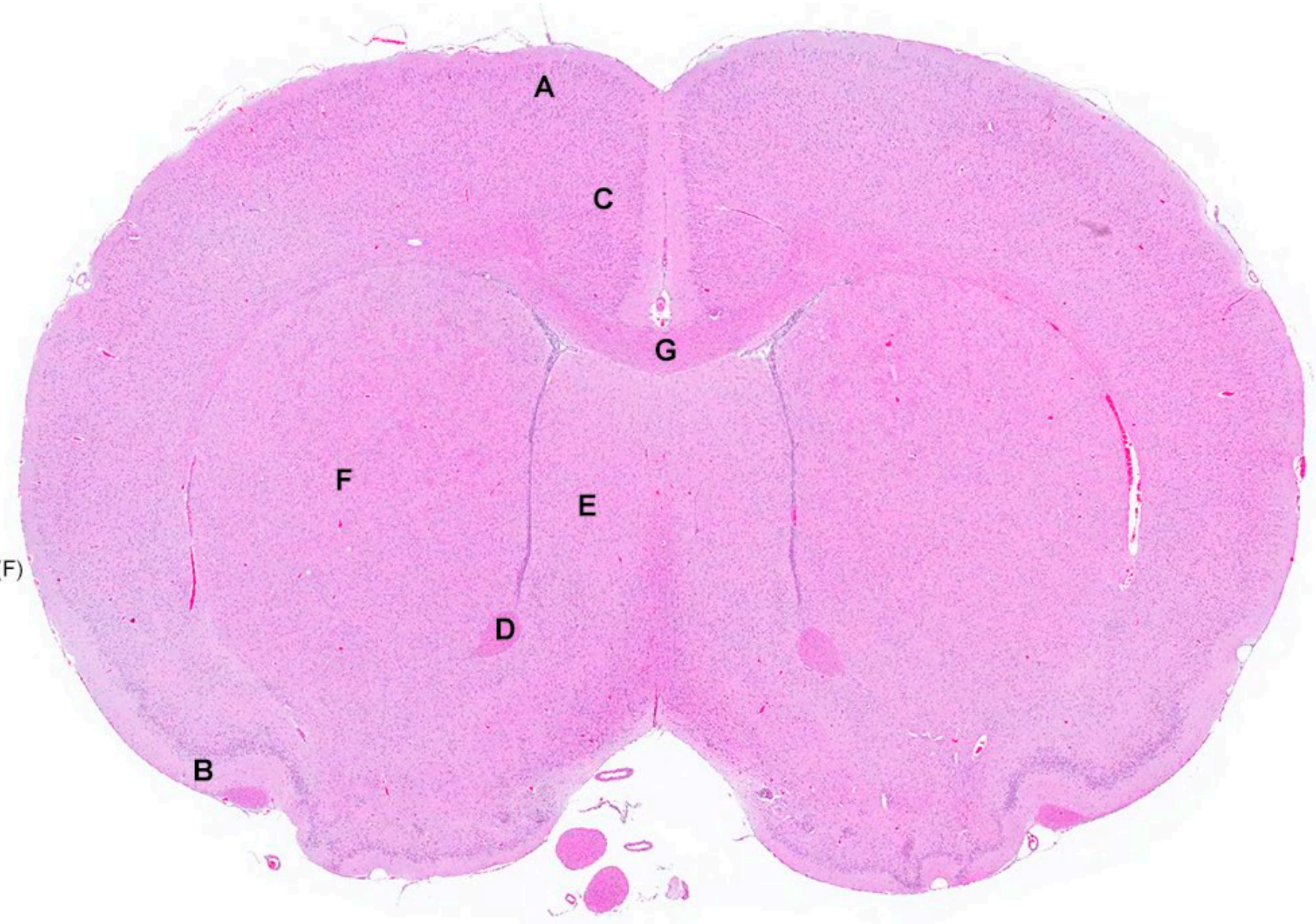
Cingulate Cortex (C)

Anterior Commissure (D)

Septal Nuclei (E)

Caudate Nuclei & Putamen (F)

Corpus Collosum (G)



NTP-7 Level 3

Parietal Cortex (A)

Amygdaloid Nucleus (B)

Hippocampal Dentate Gyrus (C)

Thalamus (D)

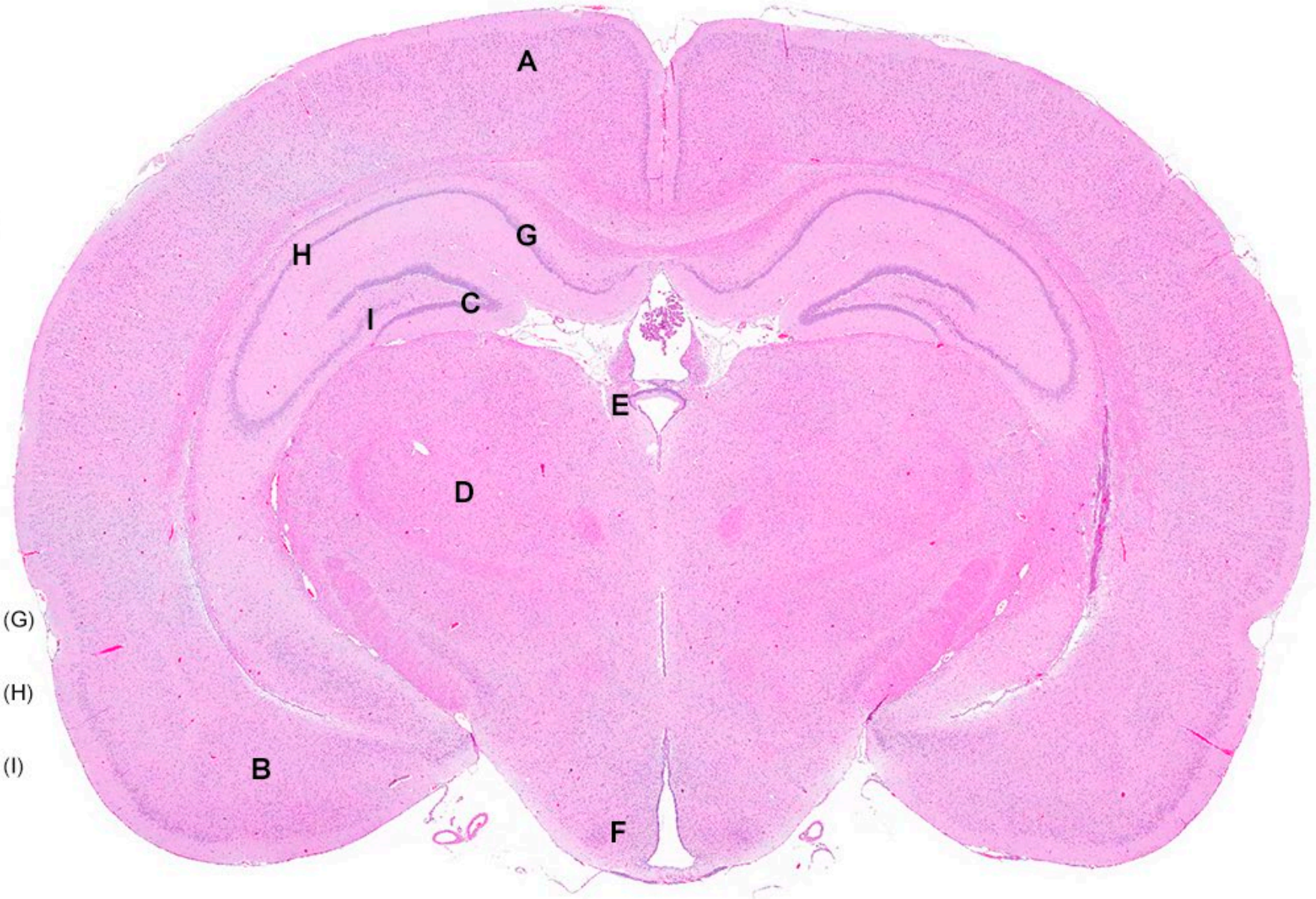
Habenular Nucleus (E)

Hypothalamus (F)

Cornu Ammonis 1 (CA1) (G)

Cornu Ammonis 2 (CA2) (H)

Cornu Ammonis 3 (CA3) (I)



NTP-7 Level 4

Anterior (Rostral) Colliculi (A)

Medial Geniculate Body (B)

Red Nucleus (C)

Raphe Nucleus (D)

Substantia Nigra (E)

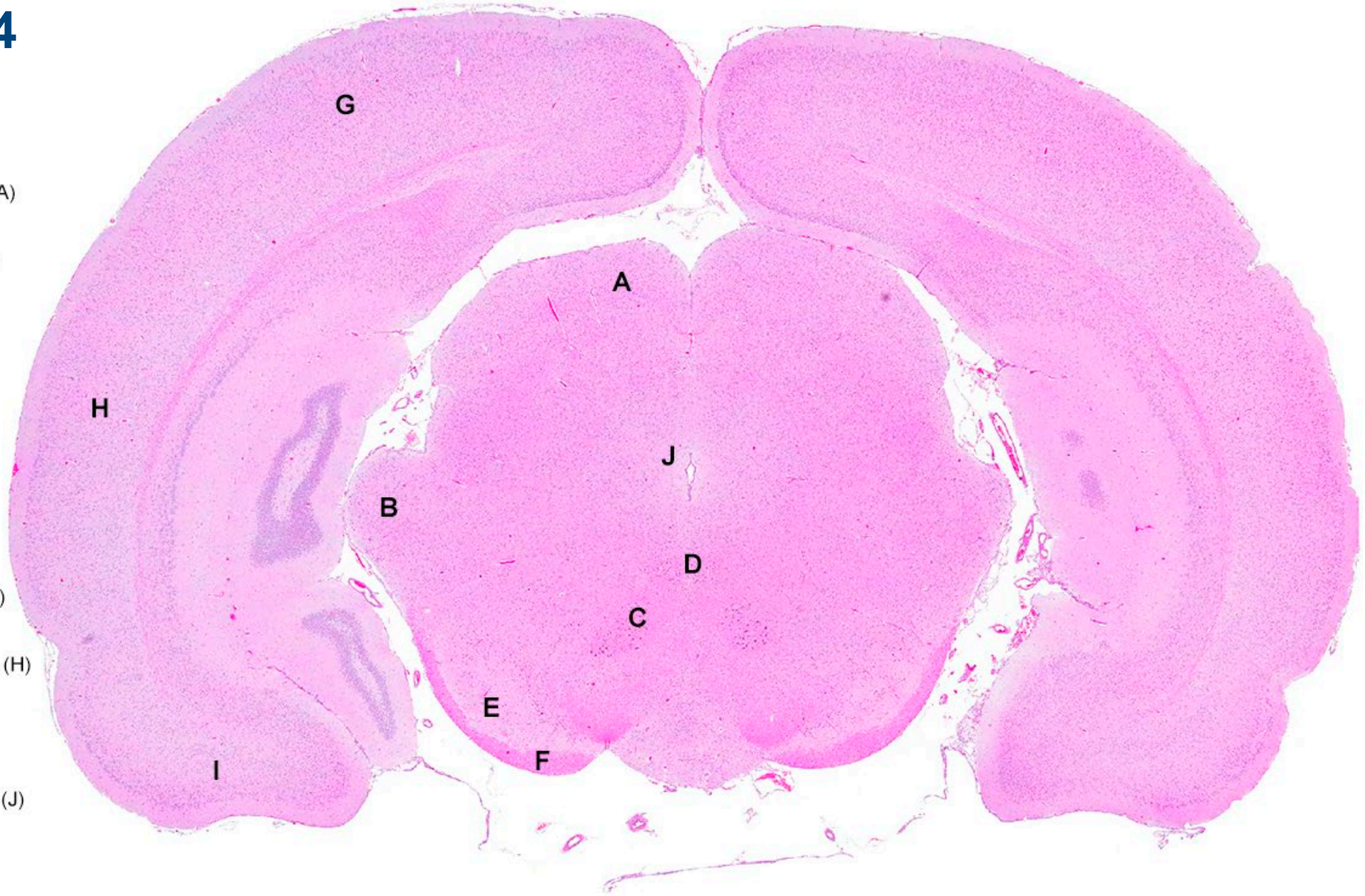
Cerebral Peduncle (F)

Occipital (Visual) Cortex (G)

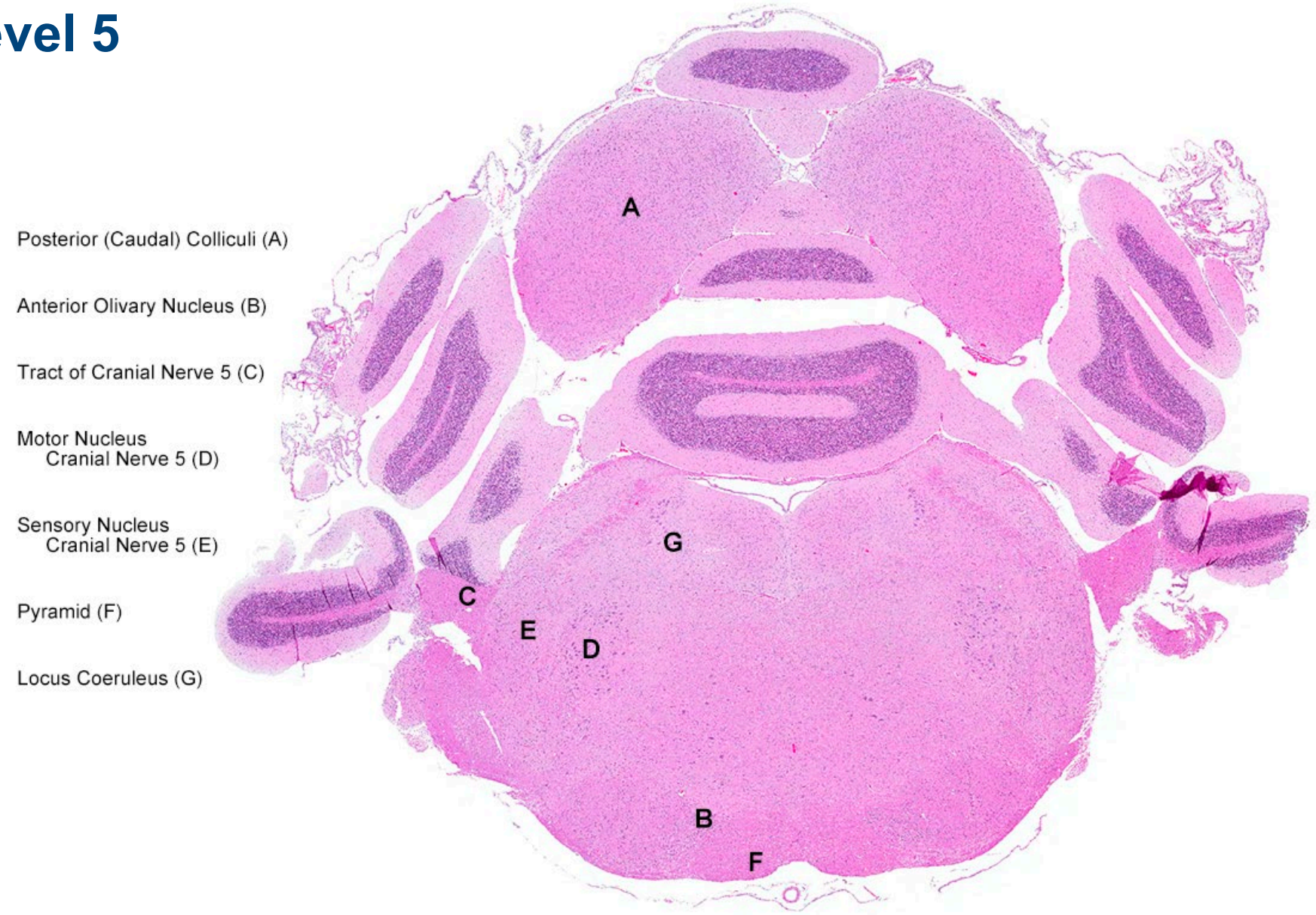
Temporal (Auditory) Cortex (H)

Entorhinal Cortex (I)

Periaqueductal Gray mater (J)



NTP-7 Level 5


Posterior (Caudal) Colliculi (A)
Anterior Olivary Nucleus (B)
Tract of Cranial Nerve 5 (C)
Motor Nucleus
Cranial Nerve 5 (D)
Sensory Nucleus
Cranial Nerve 5 (E)
Pyramid (F)
Locus Coeruleus (G)
A
G
C
E
D
B
F

NTP-7 Level 6

Vermis (A)

Ansiform Lobe (B)

Spinal Tract of Cranial Nerve 5 (C)

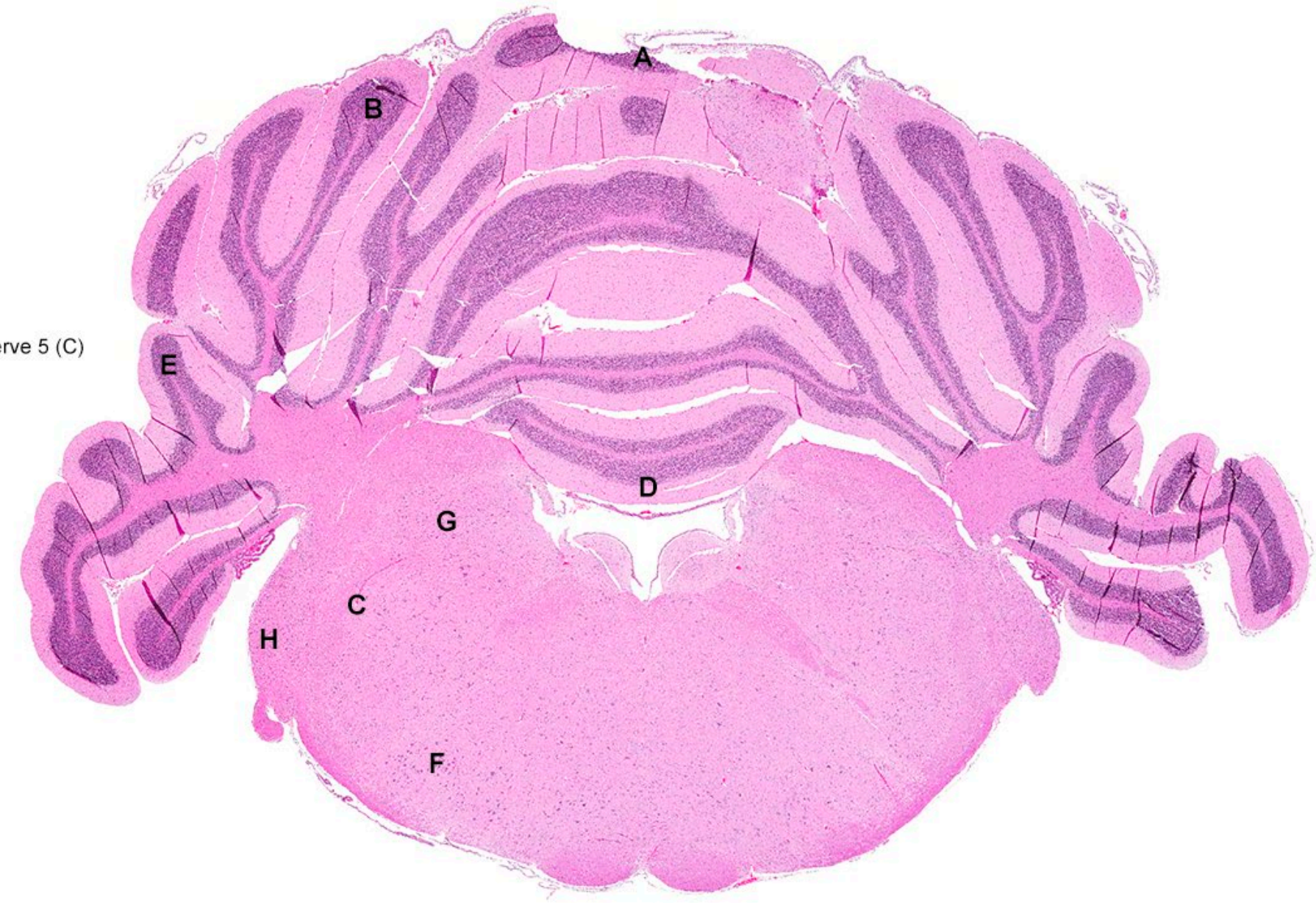
Lingula (D)

Paraflocculus (E)

Facial Nucleus (F)

Vestibular Nucleus (G)

Cochlear Nucleus (H)



NTP-7 Level 7

Cerebellar Declive (A)

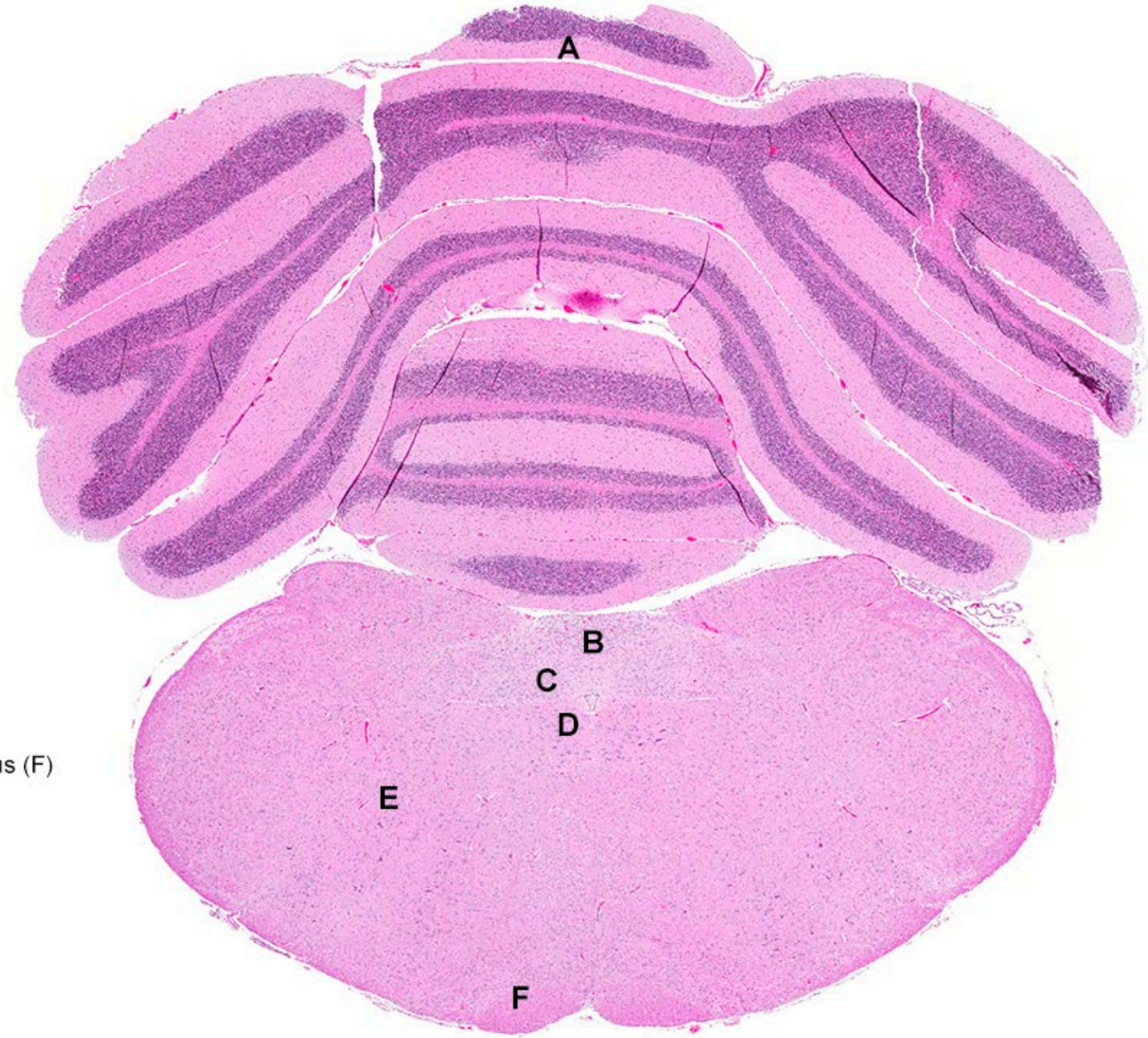
Area Postrema (B)

Cranial Nerve 10
Nucleus (C)

Cranial Nerve 12
Nucleus (D)

Reticular Nucleus (E)

Posterior Olivary Nucleus (F)

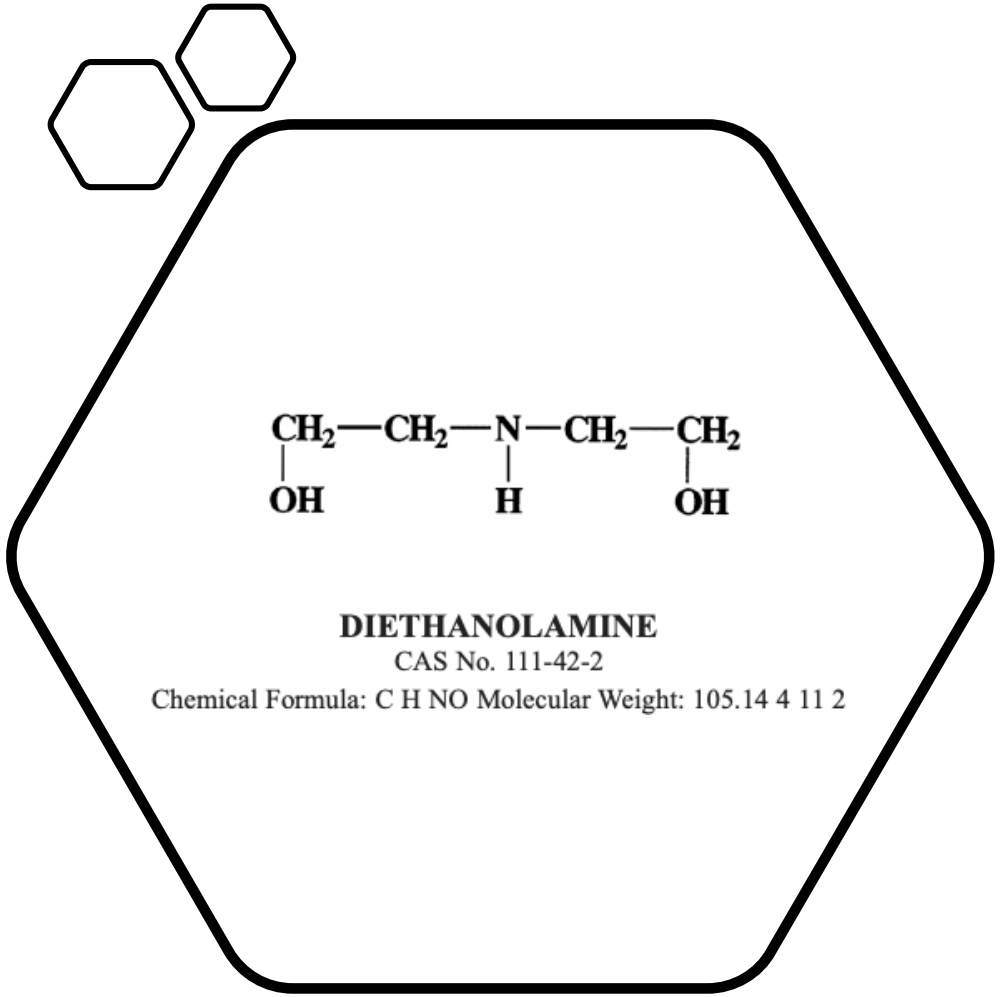


Additional references

Bolon, B., Garman, R. H., Pardo, I. D., Jensen, K., Sills, R. C., Roulois, A., Radovsky, A., Bradley, A., Andrews-Jones, L., Butt, M., & Gumprecht, L. (2013). STP position paper: Recommended practices for sampling and processing the nervous system (brain, spinal cord, nerve, and eye) during nonclinical general toxicity studies. Toxicologic Pathology, 41(7), 1028–1048. https://doi.org/10.1177/0192623312474865 (This paper deals with rodent and non-rodents.)

#1 Diethanolamine

- Diethanolamine is used in cutting oils, soaps, shampoos, cleaners, polishers, cosmetics, and pharmaceuticals.
- It is also used as an intermediate in the rubber chemicals industry, as a humectant (absorbing agent) and softening agent, and as an emulsifier and dispersing agent in various agricultural chemicals.
- Clinical signs: Tremors & postural disturbance in rats exposed for 13 weeks
- Now review the following whole slide images: Figure 1-1 and Figure 1-2.



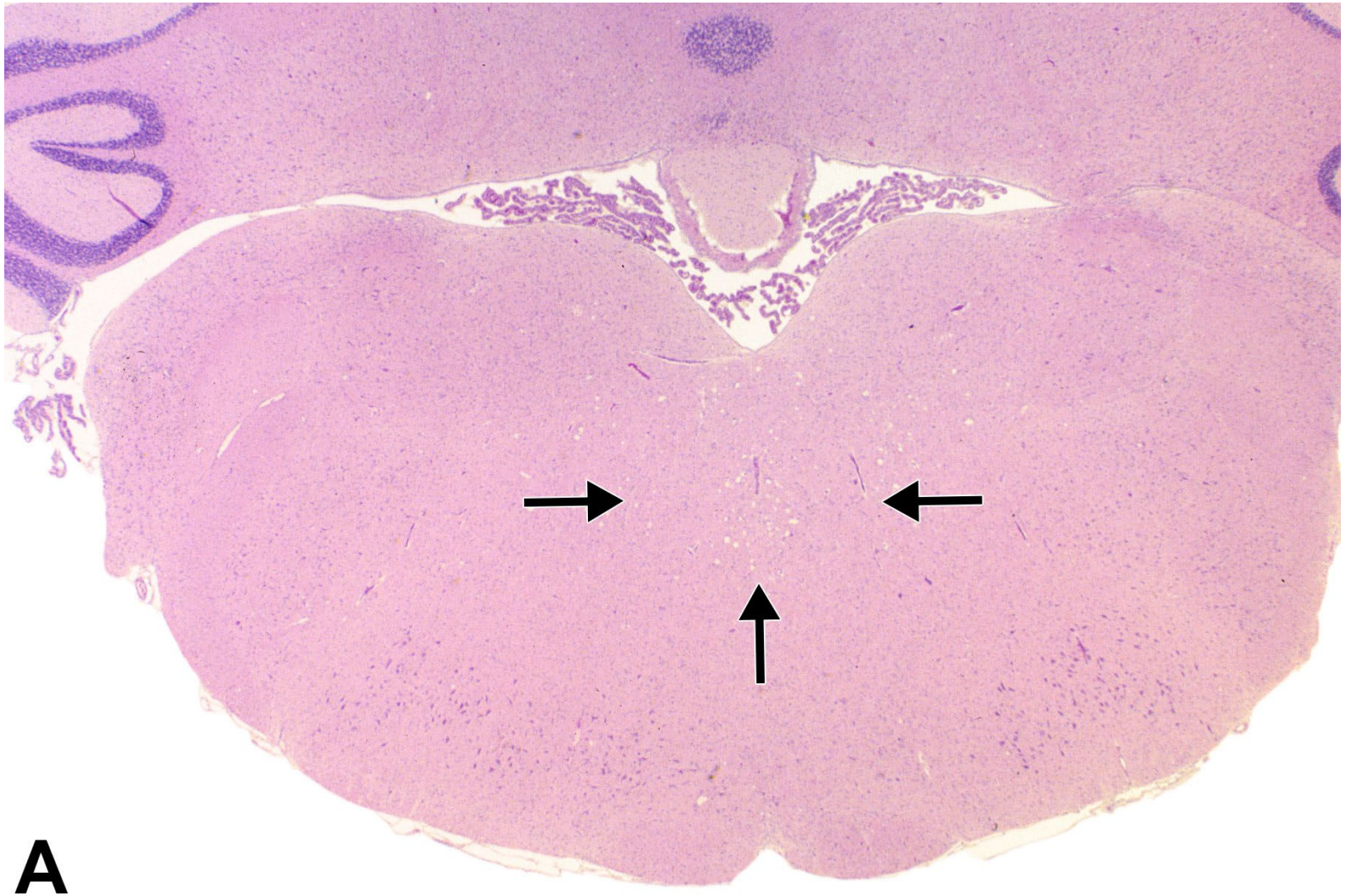
Diethanolamine National Toxicology Program, (1999): CNS Lesions

- In the brain of treated male and female rats there was consistent micro- and macrospongiosis in the medulla oblongata associated with the medial longitudinal fasciculus fibers and fibers of the dorsal tegmentum (Figure 1A and Figure 1B).
- The medial longitudinal fasciculus carries information about eye movement. It connects the cranial nerve nuclei III (oculomotor nerve), IV (trochlear nerve) and VI (abducens nerve) together, and integrates movements directed by the gaze centers (frontal eye field) and information about head movement (from cranial nerve VIII, vestibulocochlear nerve). It is an integral component of saccadic eye movements as well as vestibulo-ocular and optokinetic reflexes.
- Occasionally diethanolamine involved fibers from the genu of the 7th (facial) and the 8th (vestibulocochlear) nerves.
- Closer examination in many cases indicated that vacuoles contained either wisps of degenerate cytoplasm or rarely swollen granular axoplasmic material (Figure 1C; spinal cord). Several convincing examples of degenerate and necrotic macrophages were also visible in some of the vacuoles.

Diethanolamine National Toxicology Program, (1999): CNS Lesions

- The vacuolar spaces that were evident suggested earlier axonal swelling prior to degeneration and loss. This is a common sequence of events in axonal injury in the CNS and PNS. In some instances, there were small aggregates of astrocytic cells adjacent to affected areas.
- No evidence of neuronal necrosis or degeneration was seen in nuclei of the medulla.
- These consistent spongy lesions in the medulla were not considered artifact because of the associated degenerative and astrocytic reactive changes. However, rather than a primary demyelination lesion, these lesions appeared to indicate site-specific axonal injury with associated degeneration of the surrounding myelin.

Figure 1A. Medulla. Macrospongiosis (arrows) of the medial longitudinal fasciculus fibers and dorsolateral reticular gray matter. Female rat exposed to 2.5 mg/kg diethanolamine for 13 weeks. H&E, 4.0X.



A

Figure 1B. Medulla. Higher magnification of macrospongiosis and axonopathy of the medial longitudinal fasciculus fibers and dorsolateral reticular gray matter in Figure 1A. Note the small aggregates of astrocytic cells adjacent to areas of macrospongiosis. The smallest spongy foci represent microspongiosis of the neuropil and are difficult to see at lower magnifications. Female rat exposed to 2.5 mg/kg diethanolamine for 13 weeks. H&E, 80X

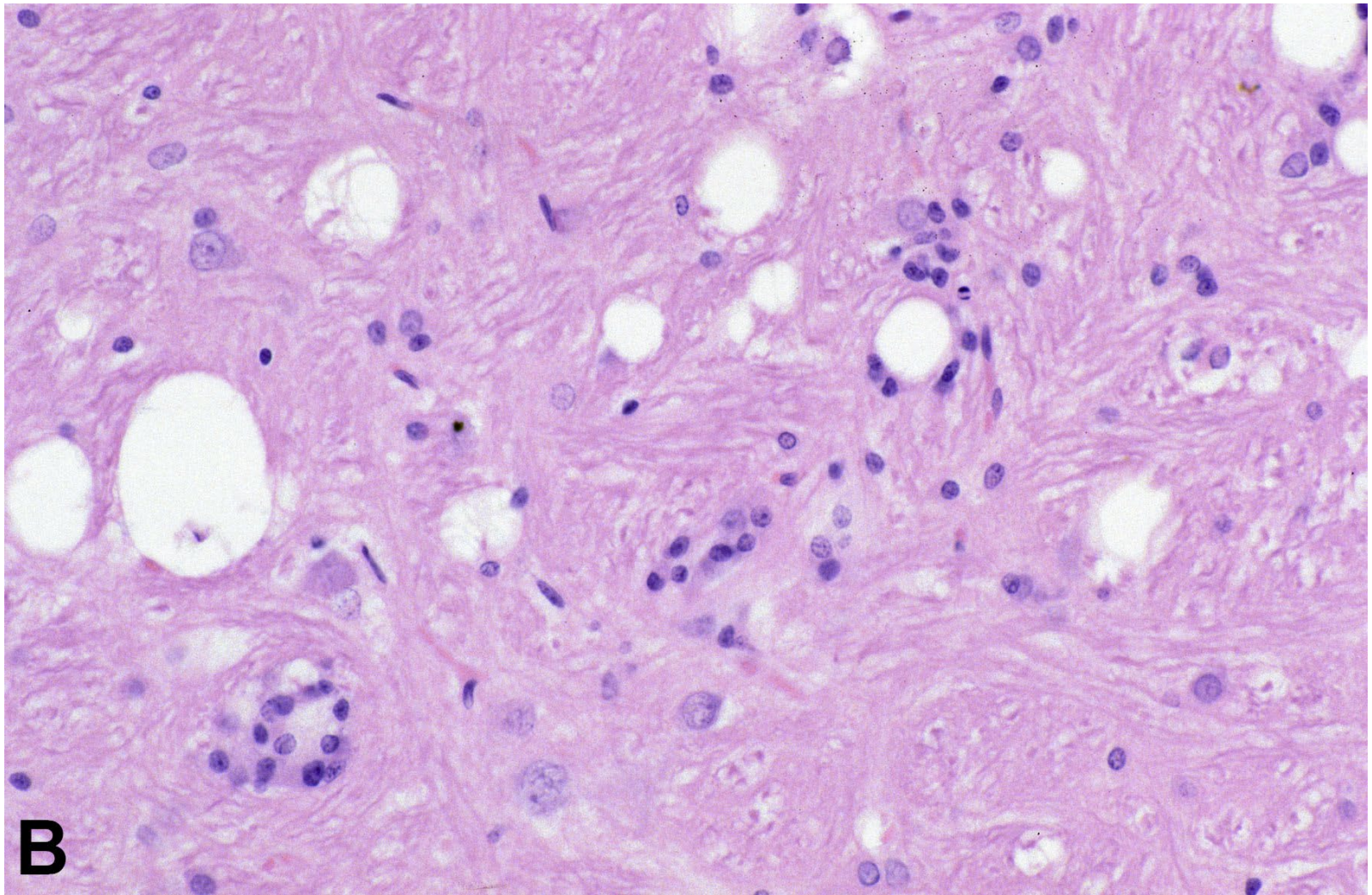
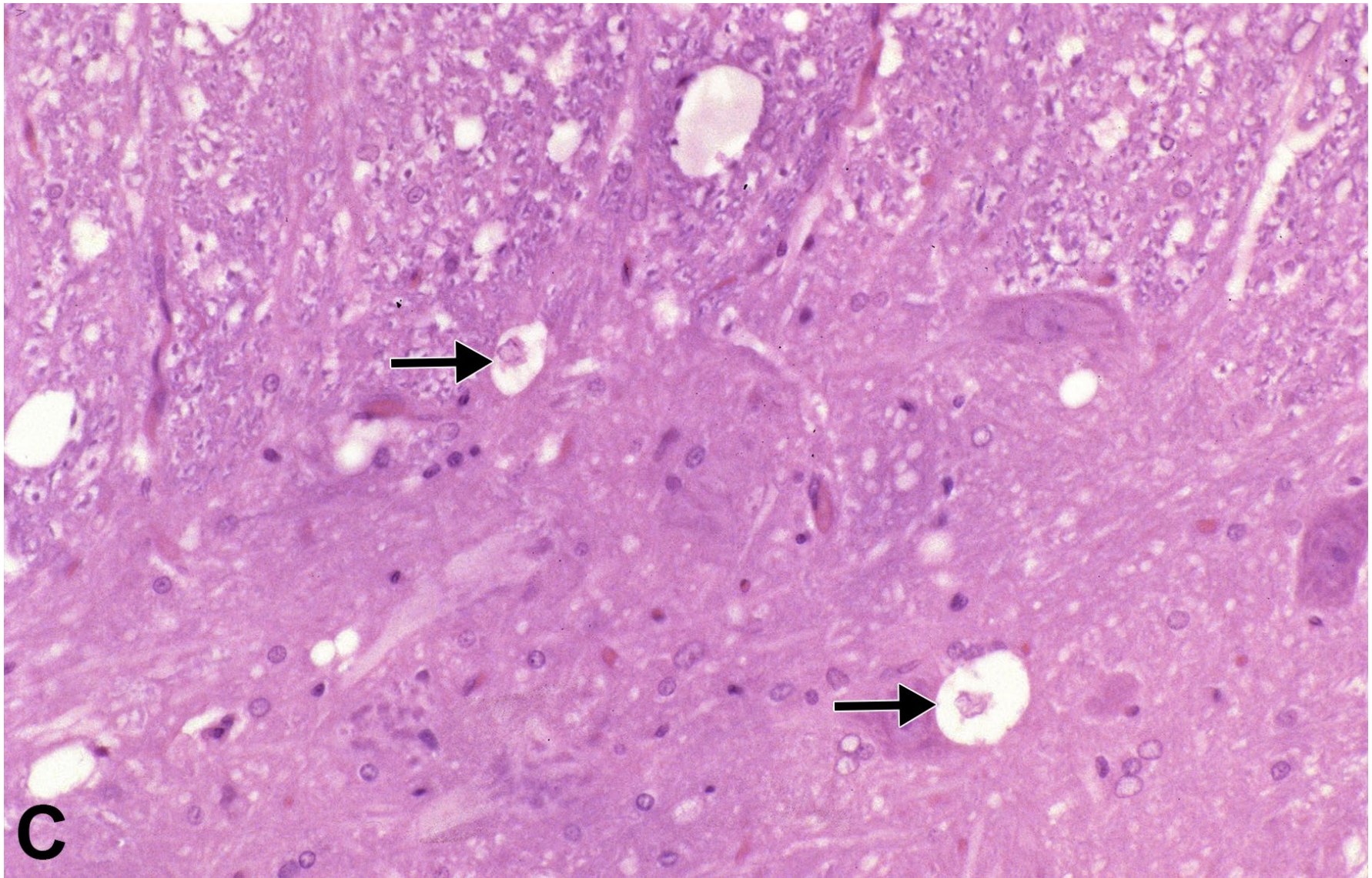


Figure 1C. Spinal Cord. Note axonal bodies within vacuoles (arrows). Male rat exposed to 5.0 mg/kg diethanolamine for 13 weeks. H&E, 80X.

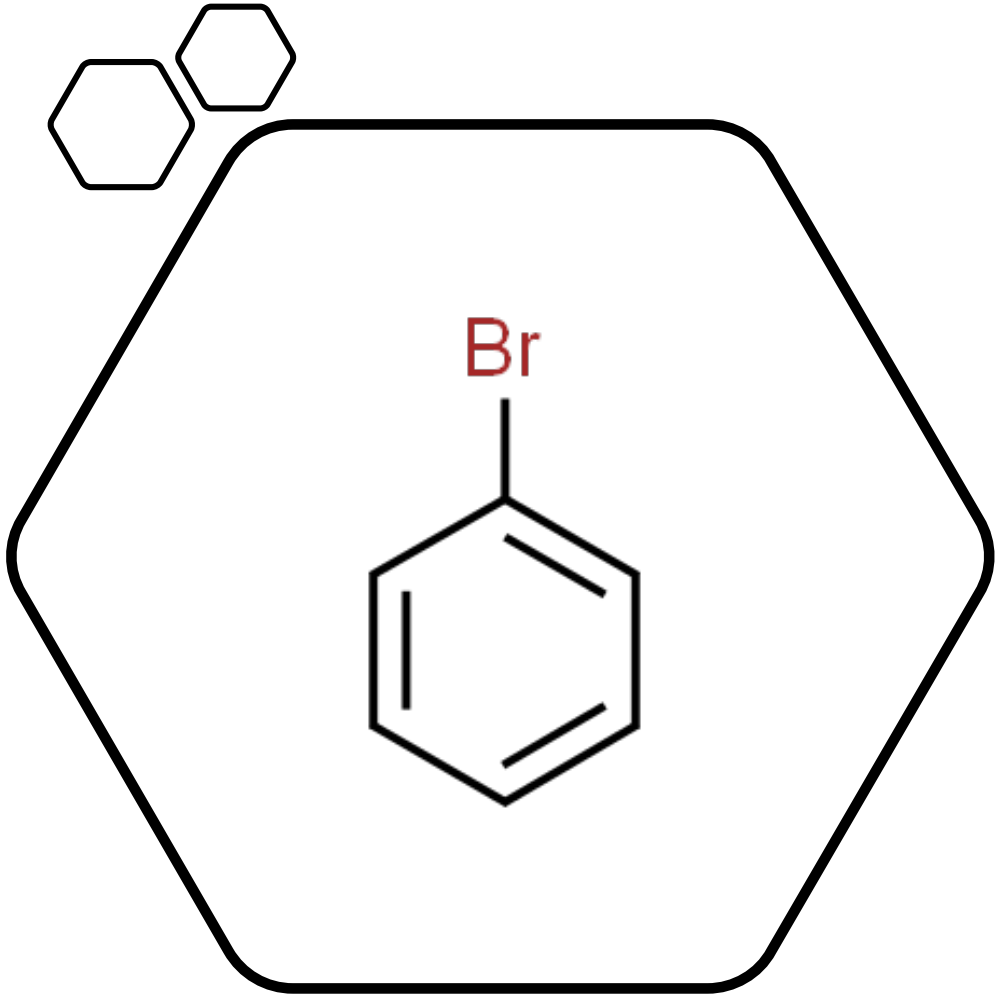


#1 Diethanolamine Neuropathological diagnosis

- Medulla, (Medial longitudinal fasciculus, Cranial nerves 7 & 8), neuron, axonopathy

#2 Bromobenzene

- Release of bromobenzene to the environment may occur during its production and the production of phenyl magnesium bromide, as well as in its use as a solvent and as an additive in motor oil (Hazardous substance data bank HSDB, 2003).
- It has been detected at low frequencies and at low concentrations in samples of food, ambient air, and finished water.
- Clinical signs: Ataxia & hypoactivity in rats
- Now review the following whole slide images: Figure 2-1, Figure 2-2, Figure 2-3, and Figure 2-4.



Bromobenzene CAS No. 108-86-1: CNS Lesions

- Examination of brains from high-dose male F344/N rats indicated neuronal injury in many regions with more prominent lesions in some of those locations.
- Most prominent was acute necrosis of the cerebellar internal granule cells with approximately 30-60% affected in many folia of the one cerebellar section available for examination in each case (Figure 2A; cerebellum and Figure 2B; piriform cortex). In one section, more chronic lesions of mineralization of the folial internal granule cell layer were evident and acute necrotic cells were absent. Internal granular cell necrosis should be distinguished from artifact by the focal to multifocal nature of the lesion and lack of other autolytic features in the former case.
- Mineralization appeared as laminated spheroids (calcospherites). Focal mineral deposits were also seen in the olivary nucleus and in the neuropil.
- In one section, there was vacuolation of the cerebellar roof nuclei neuropil.
- There were some rare examples of double and triple astrocytic nuclei occurring in the cerebral cortex.

Bromobenzene CAS No. 108-86-1: CNS Lesions

- Some cortical neurons had acute metabolic arrest changes with basophilic stippling of the nuclei and eosinophilic contracted cytoplasm (so-called “ischemic, eosinophilic, neuronal necrosis”).
- Many examples of pyknotic nuclei were evident in the cortex which appeared to be necrotic glial cells.
- This change must be distinguished from the rather widespread artifactual basophilic neuronal change that was present in slides from controls and treated groups alike.
- Specific sites of neuronal injury and obvious cell death were seen in the piriform cortex, cerebral cortex (in a bilaterally symmetrical pattern), thalamus, olivary nucleus and tegmental neurons.

Figure 2A. Cerebellum. Internal granule cell layer necrosis with mineralization forming calcospherites (arrow). Female rat exposed to 600 mg/kg bromobenzene for 13 weeks. H&E, 80X.

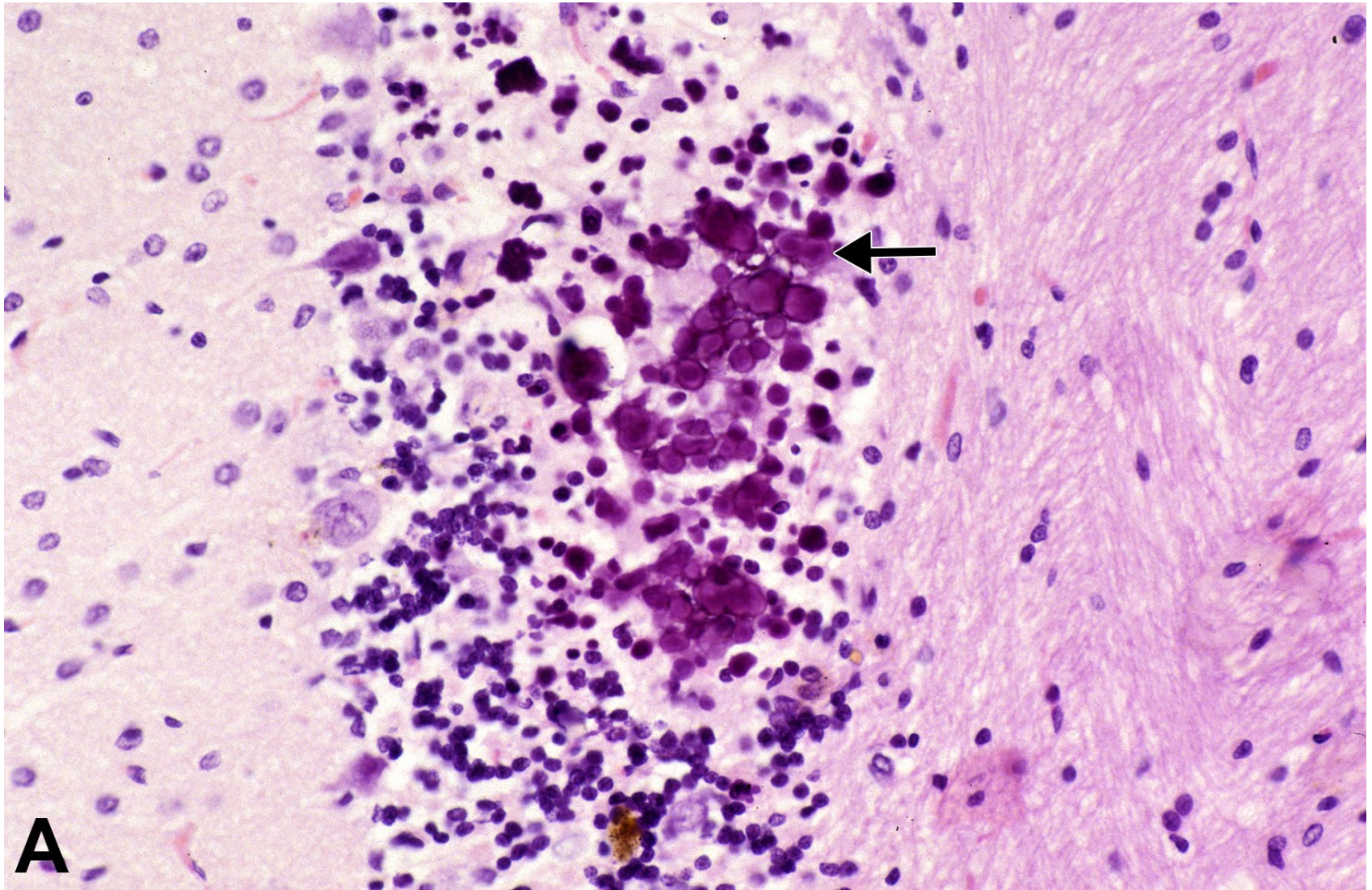
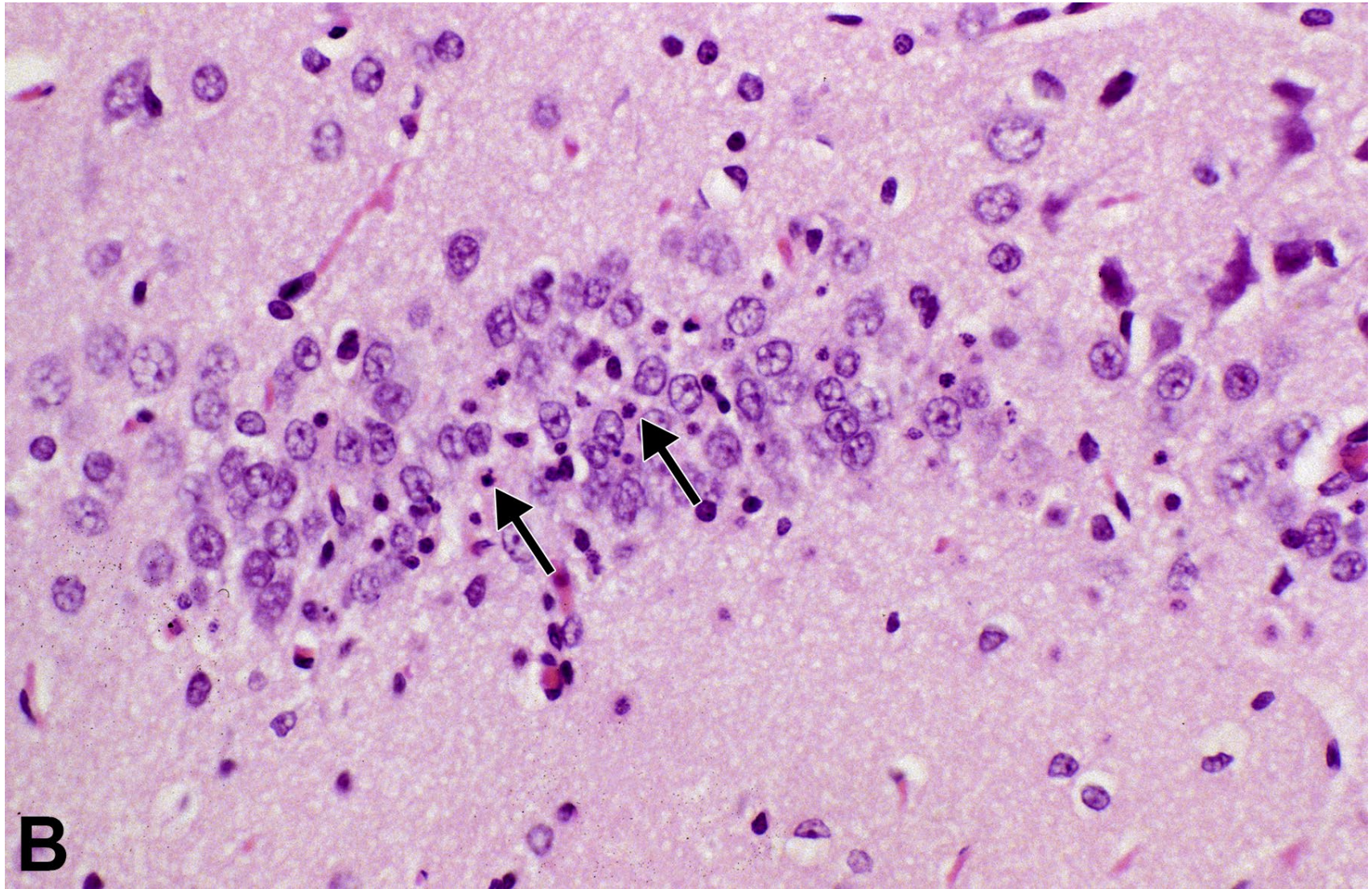


Figure 2B. Cerebrum. Piriform cortex neuronal necrosis. Note necrotic neurons with pyknotic nuclei (arrows) arranged between surviving neurons. Male rat exposed to 600 mg/kg bromobenzene for 13 weeks. H&E, 100X.

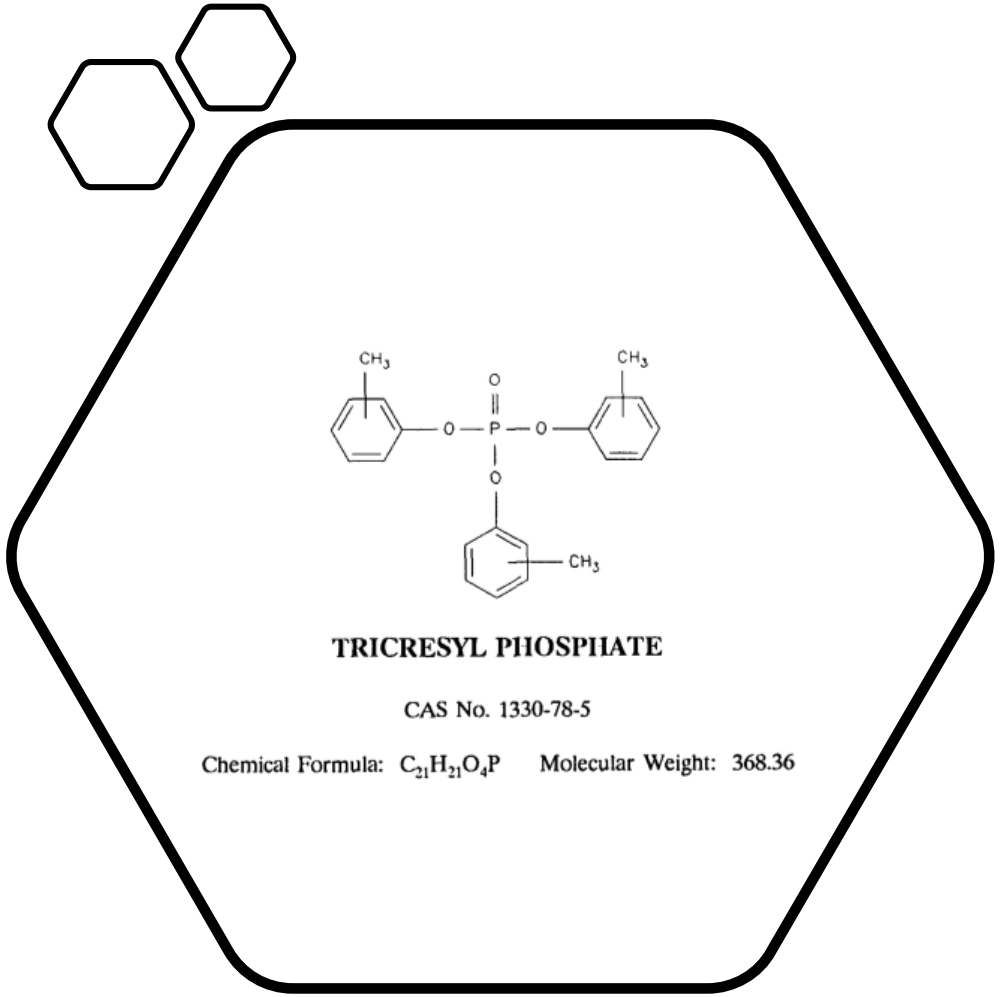


#2 Bromobenzene Neuropathological diagnoses

- Cerebellum, internal granular cell, neuron, necrosis.
- Piriform cortex, cerebral cortex, thalamus, olivary nucleus, tegmentum; neuron, necrosis

#3 Tricresyl Phosphate

- The DNTP toxicology and carcinogenesis studies were conducted by administering a mixed isomer preparation of 79% tricresyl phosphate esters (21% tri-*m*-cresyl phosphate, 4% tri-*p*-cresyl phosphate, <1% tri-*o*-cresyl phosphate, and other tricresyl phosphate esters). (TR-433)
- The ortho isomer demonstrates high toxicity due to a metabolite, 2-(ortho-cresyl)-4H-1,3,2-benzodioxaphosphoran-2-one (CBDP), which is an irreversible inhibitor of human butyrylcholinesterase and acetylcholinesterase.
- Tri-*o*-cresyl phosphate poisoning is characterized by numbness of the legs and hands accompanied by weakness or paralysis. Symptoms usually only occur after a latency period of 2-3 days.
- Clinical signs: Tremors in mice
- Now review the following whole slide images: Figure 3-1, Figure 3-2, and Figure 3-3.



Tricresyl Phosphate National Toxicology Program, (1994): CNS/PNS Lesions

- Examination of B6C3F1 mice indicated prominent neural lesions which were primarily axonal and of a particular distribution in treated male and female mice. In the CNS, these were observed as variably-sized eosinophilic spheroids (axonal bodies) within the neuropil of the affected area (Figure 3A and Figure 3B).
- There did not appear to be any associated microglial or astroglial response to these lesions. In sections of the sciatic nerve, axonal degenerative lesions consisted of multiple fragments of eosinophilic axonal debris surrounded by a vacuole (the digestion chamber).
- In many of the sciatic nerve lesions there was significant hyperplasia of Schwann cells associated with the most prominent axonal lesions (Figure 3C and Figure 3D).
- The lesions were primarily noted in the sciatic nerve, the spinal funiculi (particularly the lateral), the cerebellar roof nuclei, cerebellar medullary white matter, the vestibular nuclei and the thalamic ventral posterior lateral nucleus.
- Lesions were usually bilaterally symmetrical and were equivalent in severity.

Tricresyl Phosphate National Toxicology Program, (1994): CNS/PNS Lesions

- In the thalamic lesions, microvacuoles in the neuropil were also visible, suggesting that the axonal bodies at that level had undergone dissolution leaving only the space formerly occupied by the axon.
- Bilateral posterior collicular nuclear lesions were characterized by microvacuolation of the neuropil and the presence of numerous small eosinophilic axonal bodies.
- In available sections of the tegmentum, this was noted to be accompanied by axonal bodies in the lateral lemniscus.
- The lesions seen in the sciatic nerve and spinal cord correlated with known axonopathic effects of tri-ortho-cresyl phosphate on long proprioceptive sensory fibers (Spencer & Schaumburg, 2000).
- The lesions track the injured proprioceptive fibers from the lateral funiculi to vestibular nuclei and cerebellar roof nuclei.
- Effects of axonal injury also extended to conscious proprioceptive perception via the sensory synapse points in the thalamic ventral posterior lateral nucleus.

Tricresyl Phosphate National Toxicology Program, (1994): CNS/PNS Lesions

- No lesions were evident in cerebellar folial or cerebral cortical regions.
- One might have expected more axonal lesions in the dorsal funiculi of the spinal cord. However, in most spinal cord sections taken at necropsy in this study the cord was taken too far posterior for the dorsal funiculi to be well represented anatomically.
- The affected lateral funiculi, are partially composed of the dorsal and ventral spinocerebellar tracts which are major proprioceptive tracts subserving the thoracic, lumbar and sacral proprioceptive afferents.
- The collicular nuclear and optic radiation lesions indicated injury by tricresyl phosphate to the auditory reflex center and the visual pathway, regions not generally recognized as vulnerable to the axonopathic effect of this compound.

Figure 3A. Cerebellum. Rostral peduncle axonopathy characterized by eosinophilic spheroids (arrow). Female mouse exposed to 4200 mg/kg tricresyl phosphate for 13 weeks. H&E, 100X.

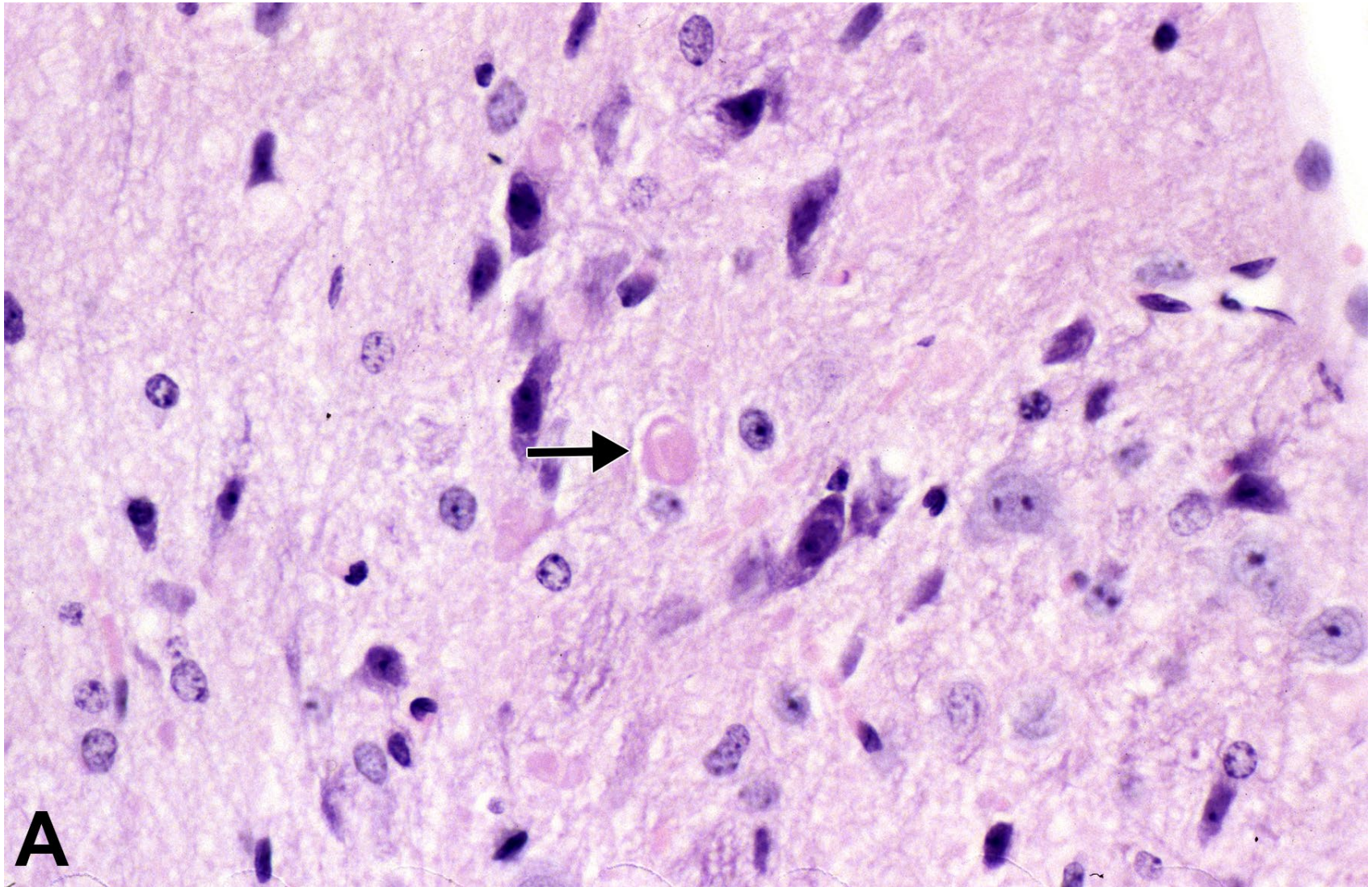


Figure 3B. Midbrain. Posterior collicular nucleus. Note presence of axonal bodies characterized by eosinophilic spheroids (arrow). Female mouse exposed to 4200 mg/kg tricresyl phosphate for 13 weeks. H&E, 100X.

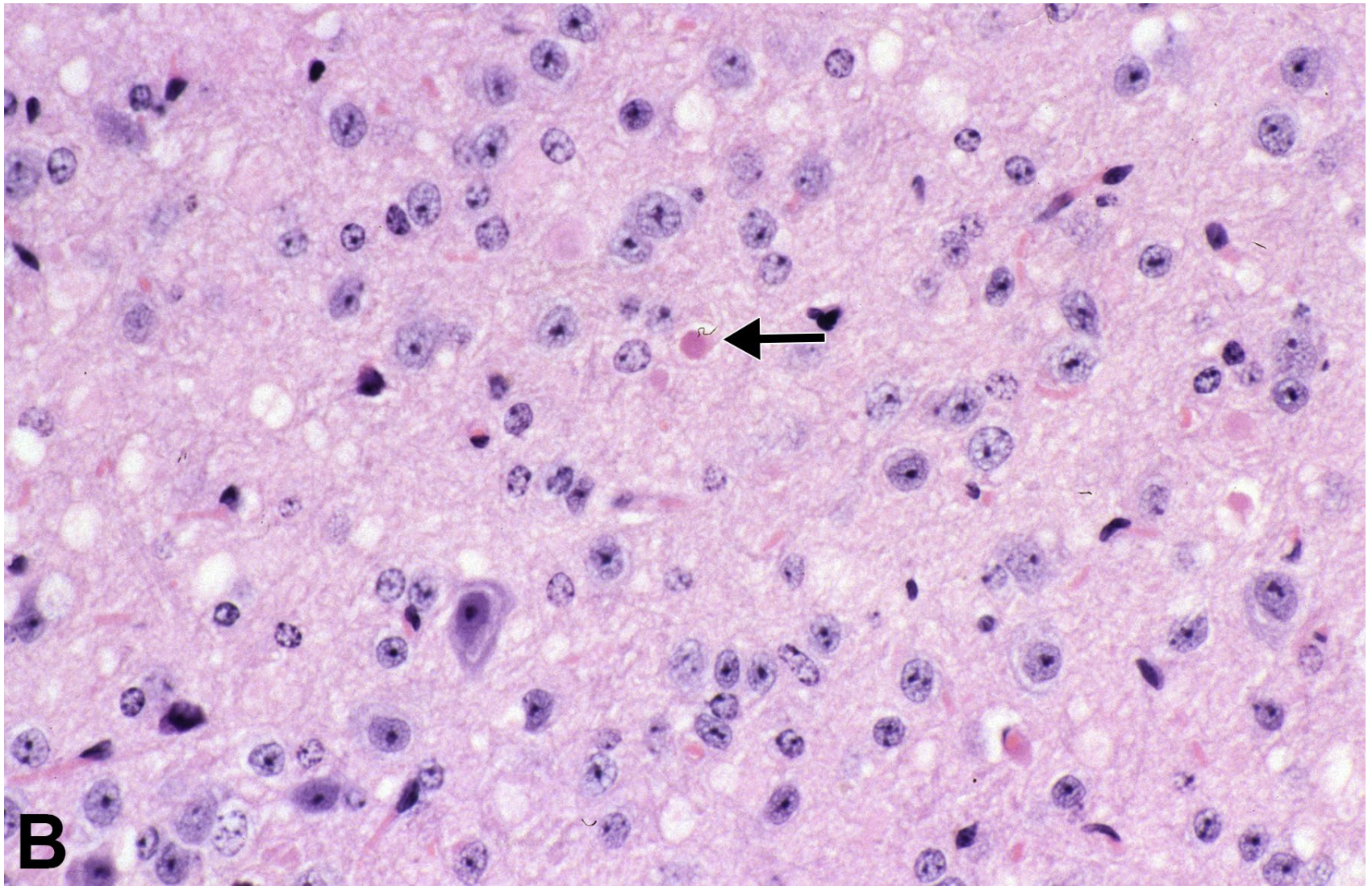


Figure 3C. Sciatic nerve. Axonopathy. Note the bands of Schwann cell hyperplasia (arrows). Male mouse exposed to 4200 mg/kg tricresyl phosphate for 13 weeks. H&E, 50X.

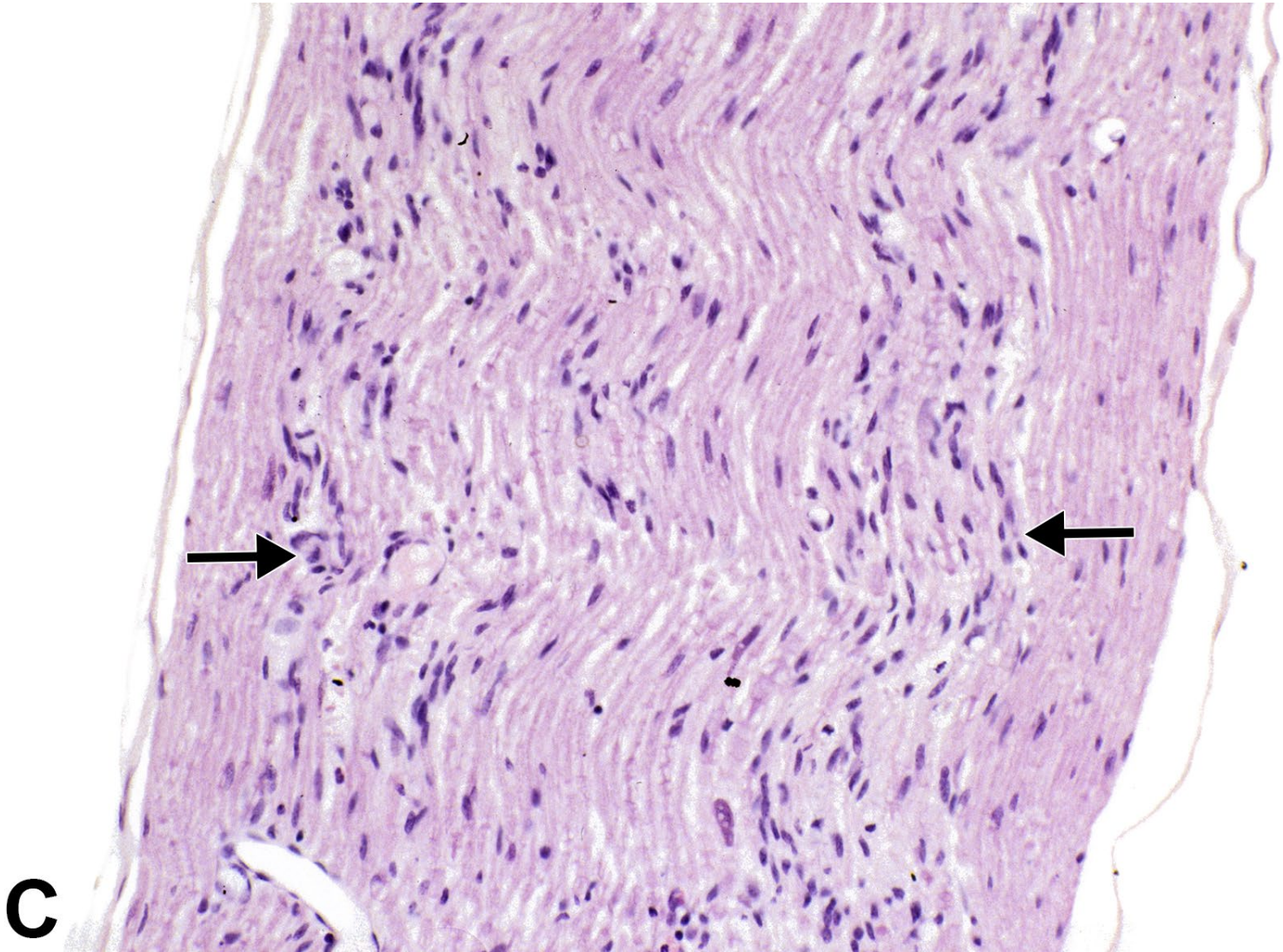
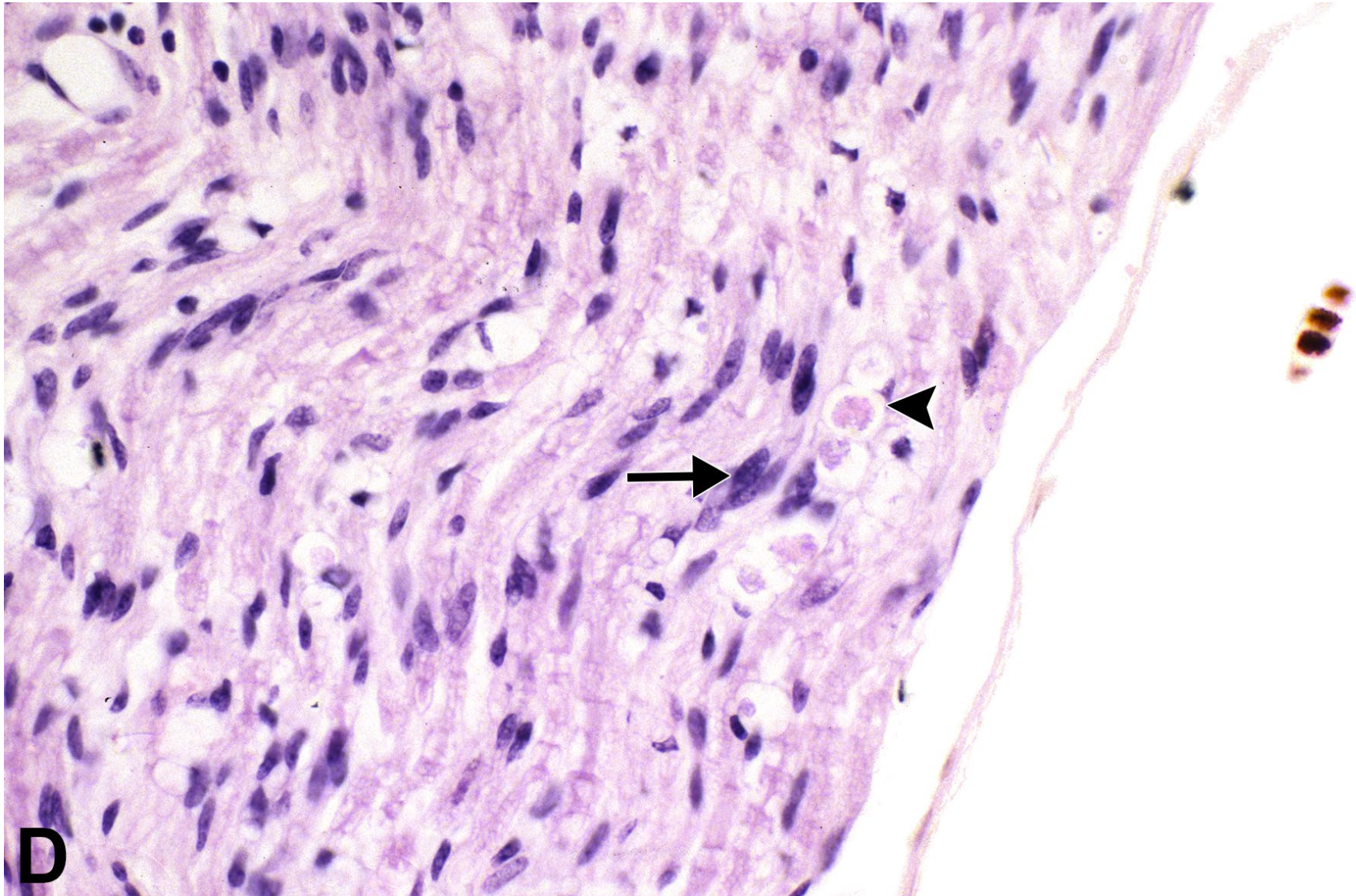


Figure 3D. Sciatic nerve. Note Wallerian degeneration (arrowhead) and Schwann cell hyperplasia (arrow). H&E, 100X.

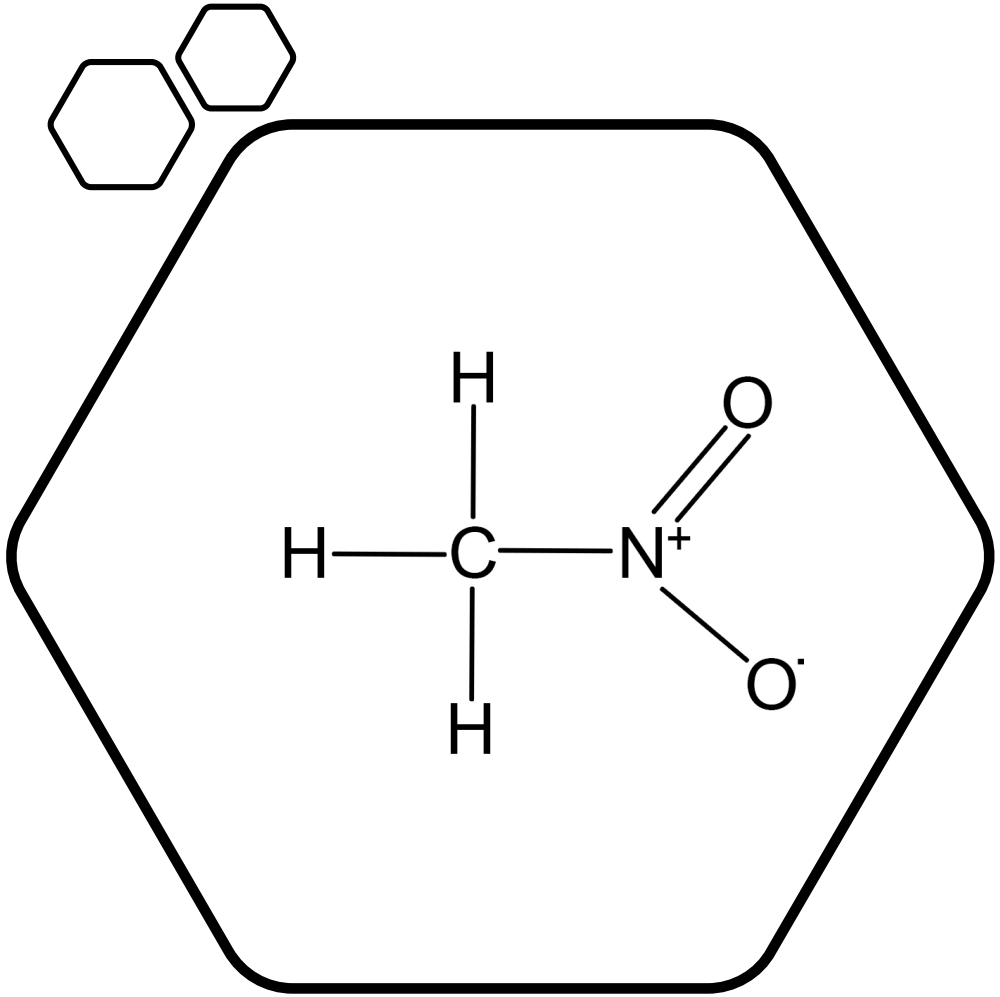


#3 Tricresyl phosphate Neuropathological diagnoses

- Sciatic nerve, spinal funiculi, cerebellar roof nuclei, cerebellar medullary white matter, vestibular nuclei, thalamic ventral posterior lateral nucleus, posterior collicular nuclei and optic radiation; neuron, axonopathy.

#4 Nitromethane

- Nitromethane has the chemical formula CH_3NO_2. It is a polar liquid used as a solvent in a variety of industrial applications – in extractions, a reaction medium, and a cleaning solvent.
- It is used in organic synthesis as an intermediate and in the manufacture of pharmaceuticals, pesticides, explosives, fibers, and coatings.
- Clinical signs: Hind limb paralysis in rats
- Now review the following whole slide image: Figure 4-1 and Figure 4-2



Nitromethane National Toxicology Program, (1997): CNS Lesions

- Lesions in the brain, spinal cord and sciatic nerve from both sexes of F344/N rats were primarily characterized by axonal degeneration in the spinal cord tracts and sciatic nerve (Figure 4A)
- The lesions were of minimal to mild severity.
- In the brain, central chromatolysis was observed in the 5th Cranial nerve nucleus (Figure 4B).
- In spinal cord sections, neuronal cell body degenerative lesions were evident and consisted of vacuolar and lytic changes (Figure 4C, Figure 4D, and Figure 4E).
- Axonal lesions affected the lateral and ventral funiculi most frequently.
- Longitudinal sections of the spinal cord made it difficult to be certain of the location of the neuronal lesions but were speculated to be most common in the ventral columns.

Figure 4A. Sciatic nerve. Note wallerian degeneration characterized by axonal swelling and fragmentation (arrows). Female rat exposed to 1500 ppm nitromethane by inhalation for 13 weeks. H&E, 100X.

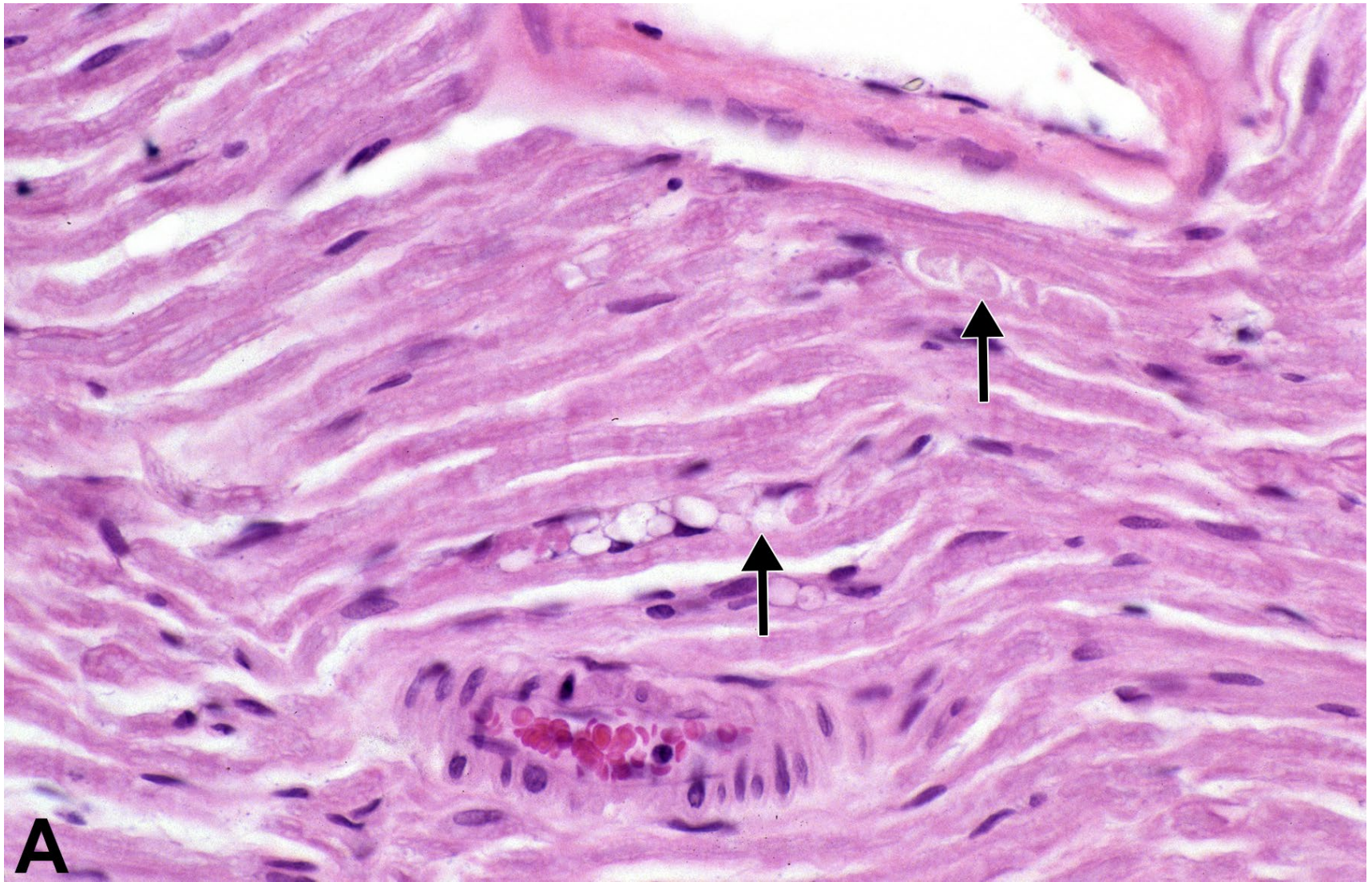


Figure 4B. Midbrain. Fifth cranial nerve motor nucleus. Note neuronal central chromatolysis and eccentric nucleus (arrows). Male rat exposed to 1500 ppm nitromethane by inhalation for 13 weeks. H&E, 80X.

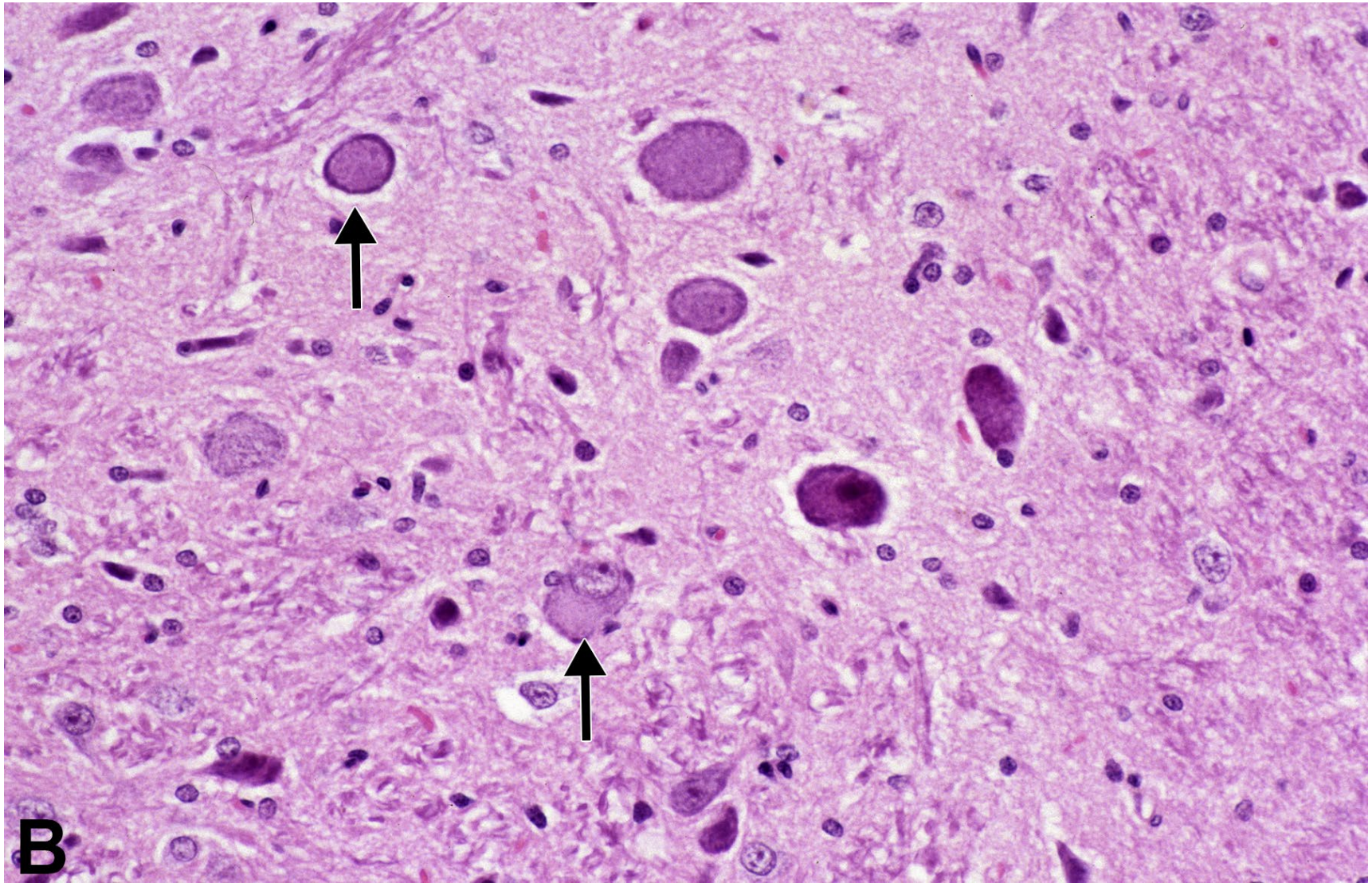


Figure 4C. Spinal cord. Note central chromatolysis (arrow). Male rat exposed to 1500 ppm nitromethane by inhalation for 13 weeks. H&E, 31.6X.

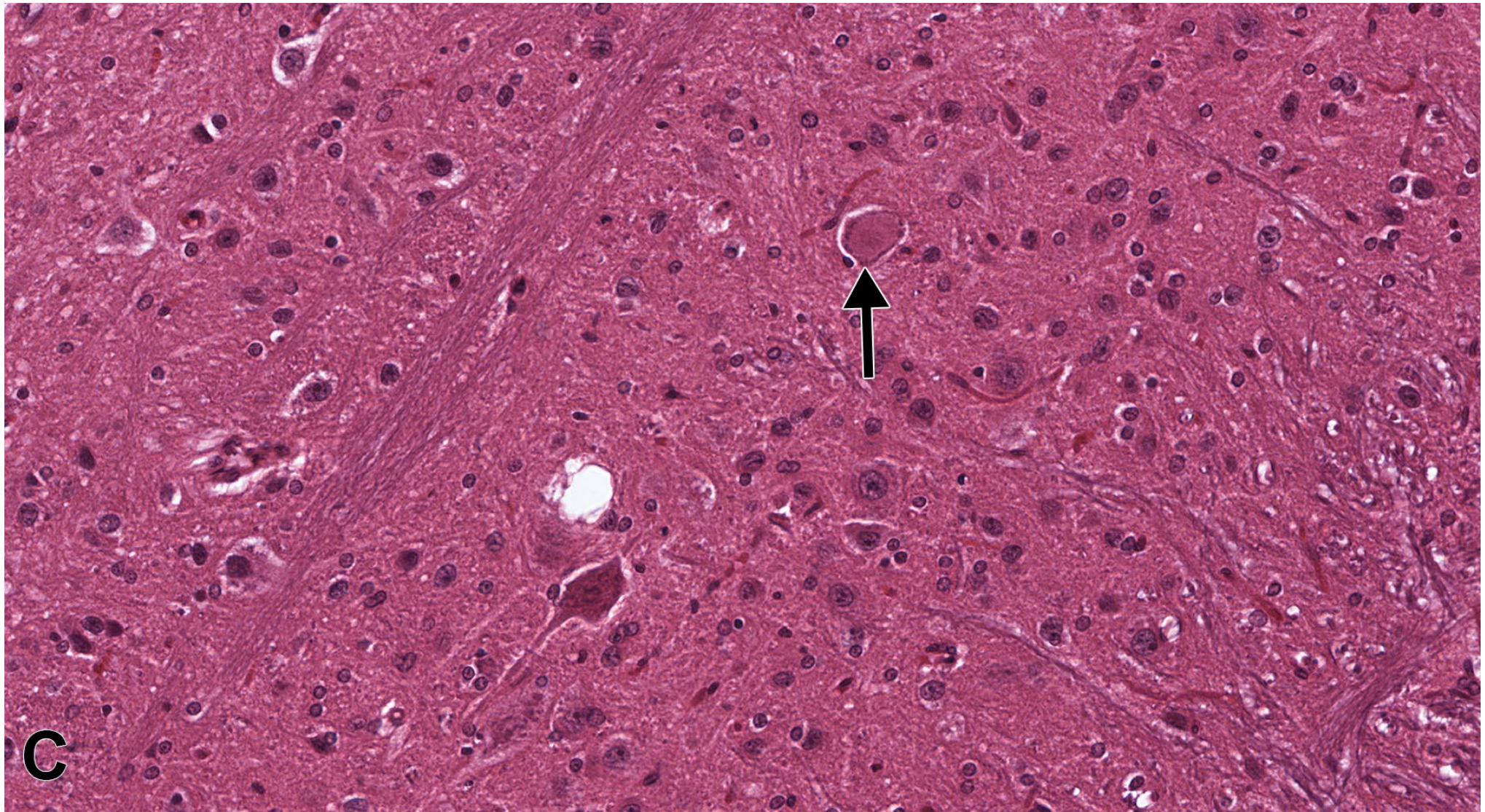


Figure 4D. Spinal cord. Note lysis of a neuron cell body (arrow). Female rat exposed to 1500 ppm nitromethane by inhalation for 13 weeks. H&E, 20X.

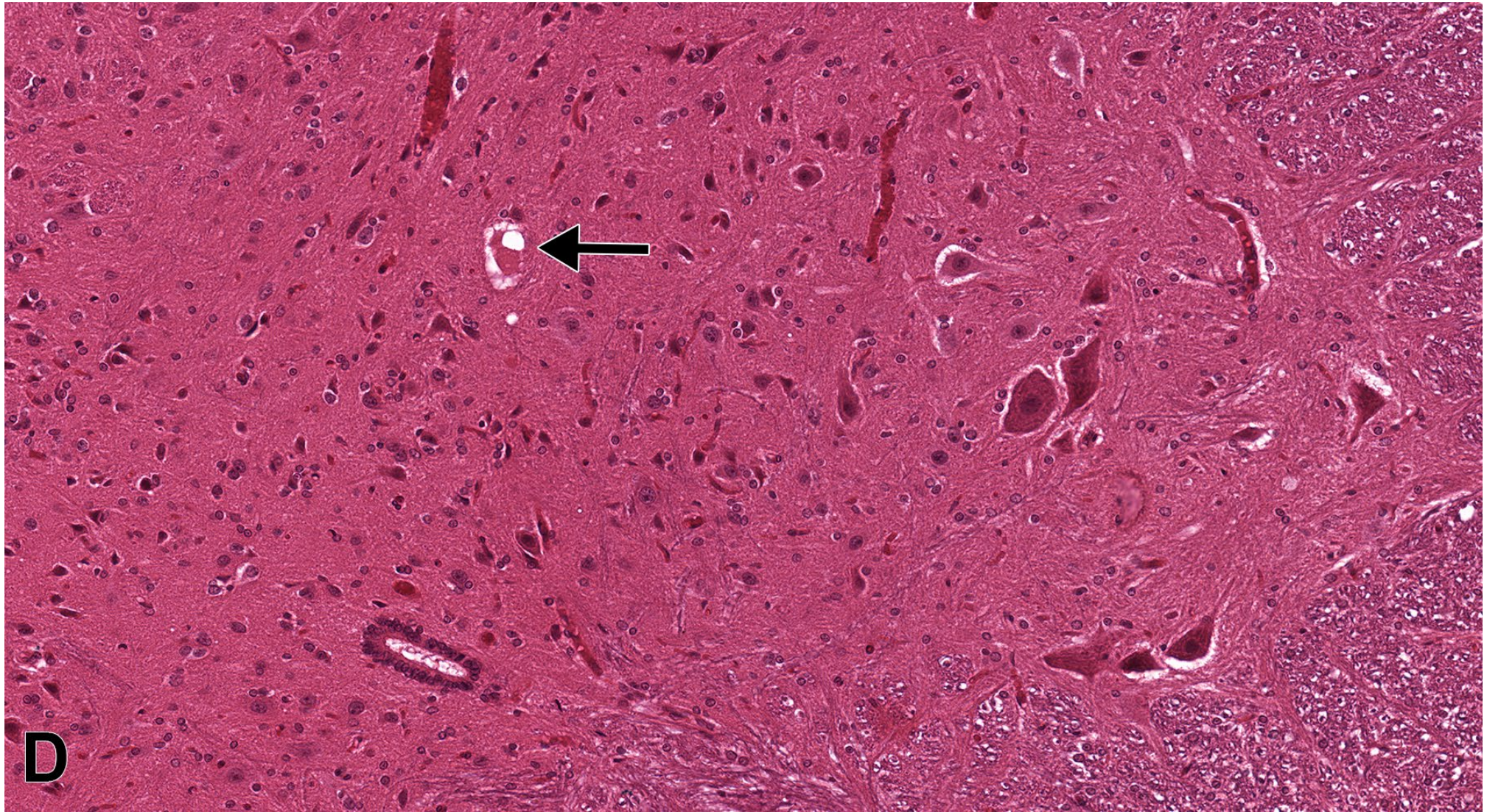
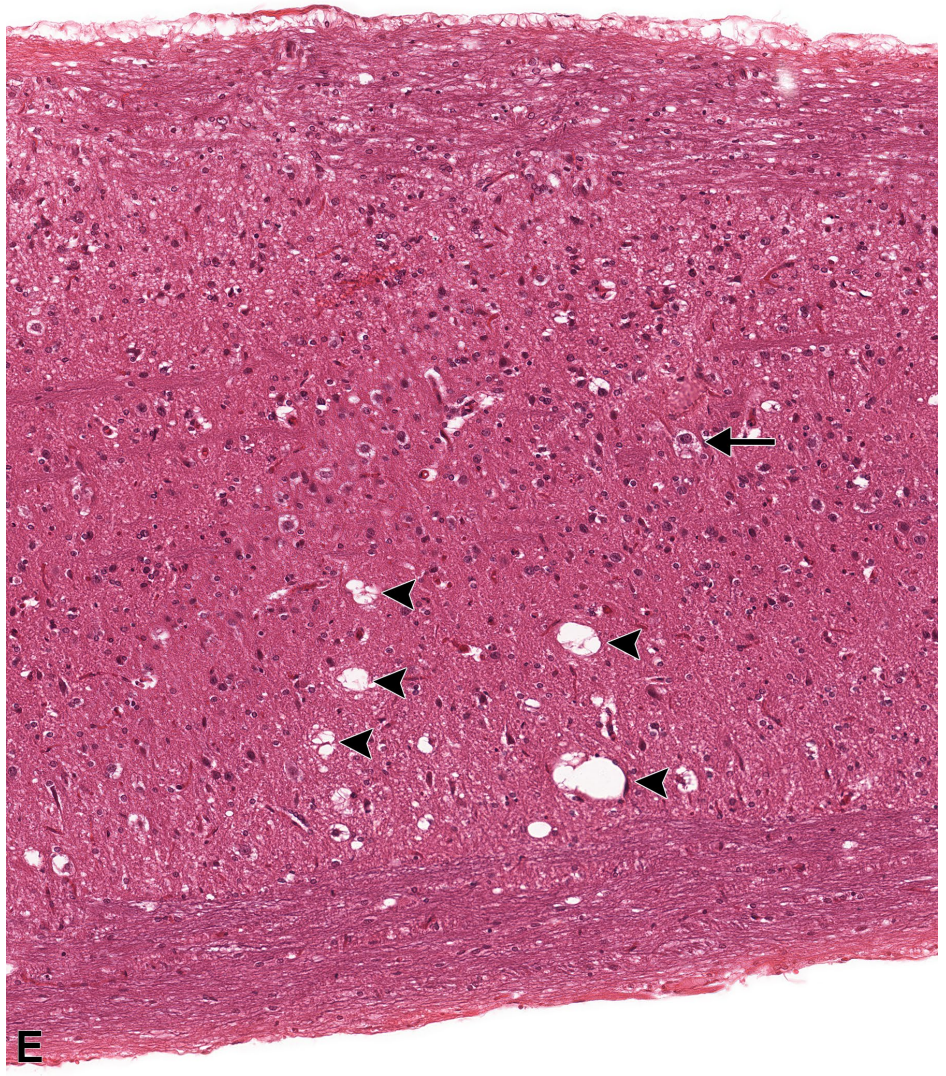


Figure 4E. Spinal cord. Note lysis of a neuron cell body (arrow) and vacuoles within the gray matter that contain cell debris (arrowheads). Female rat exposed to 1500 ppm nitromethane by inhalation for 13 weeks. H&E, original magnification 8X.

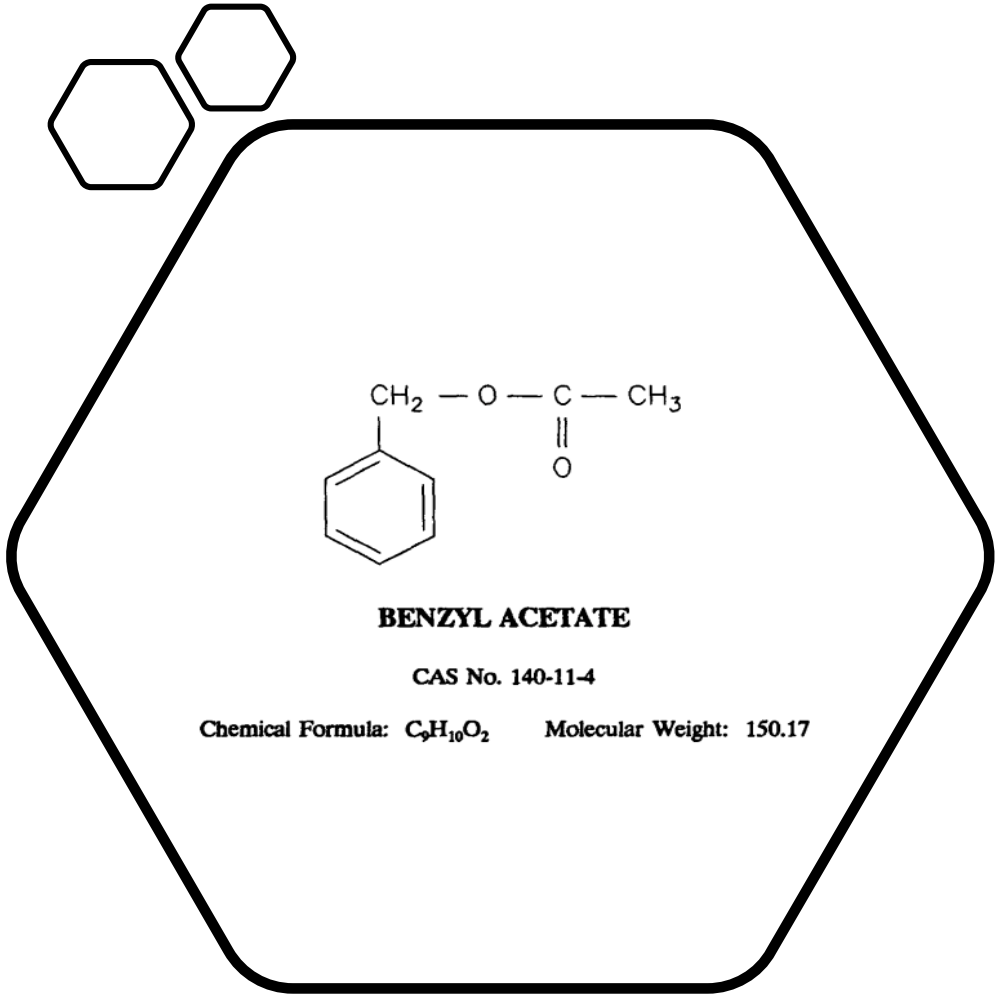


#4 Nitromethane Neuropathological diagnoses

- Fifth cranial nerve nucleus, spinal cord grey; neuron, necrosis.
- Spinal cord funiculi and sciatic nerve; neuron, axonopathy

#5 Benzyl Acetate

- Benzyl acetate is an organic ester with the molecular formula $C_9H_{10}O_2$. It is formed by the condensation of benzyl alcohol and acetic acid.
- Benzyl acetate is used to impart jasmine or apple odor to various cosmetics and personal care products like lotions, hair creams etc.
- Industrially benzyl acetate is used as a medium in extraction of plastics, resin, cellulose acetate, cellulose nitrate, oils and lacquers.
- Clinical signs: Ataxia, trembling, & sluggishness in rats
- Now review the following whole slide images: Figure 5-1, Figure 5-2, and Figure 5-3.



Benzyl acetate National Toxicology Program, (1986 A): CNS Lesions

- Fischer 344/N rats had a severe and relatively consistent hippocampal lesion that affected treated males and females.
- The primary areas affected included the dentate gyral granule cell layer neurons which were eosinophilic, shrunken and necrotic in the typical so-called “ischemic” or metabolic arrest morphology (Figure 5A, Figure 5B and Figure 5C).
- No macrophage or endothelial cell response was evident.
- Also consistent was a more scattered but focally intensive neuronal necrosis of the same type in neurons of the cornu ammonis 1 (CA1) and cornu ammonis 2 (CA2) segments of the hippocampus and sometimes cornu ammonis 3 (CA3) (Figure 5C).
- In some animals, there was a prominent gemistocytic astrocytosis in the affected hippocampal regions (also see whole slide image Figure 5-1).
- Lesions were not evident in a comprehensive review of other CNS regions. The severe hippocampal dentate gyrus and cornu ammonis (CA) regional lesions would be expected to have a major negative impact on memory processes. (Abdo 1998).

Figure 5A. Hippocampus. Dentate gyri bilateral necrosis (arrows). Female rat treated with 1000 mg/kg benzyl acetate for 13 weeks. H&E, 6.6X.

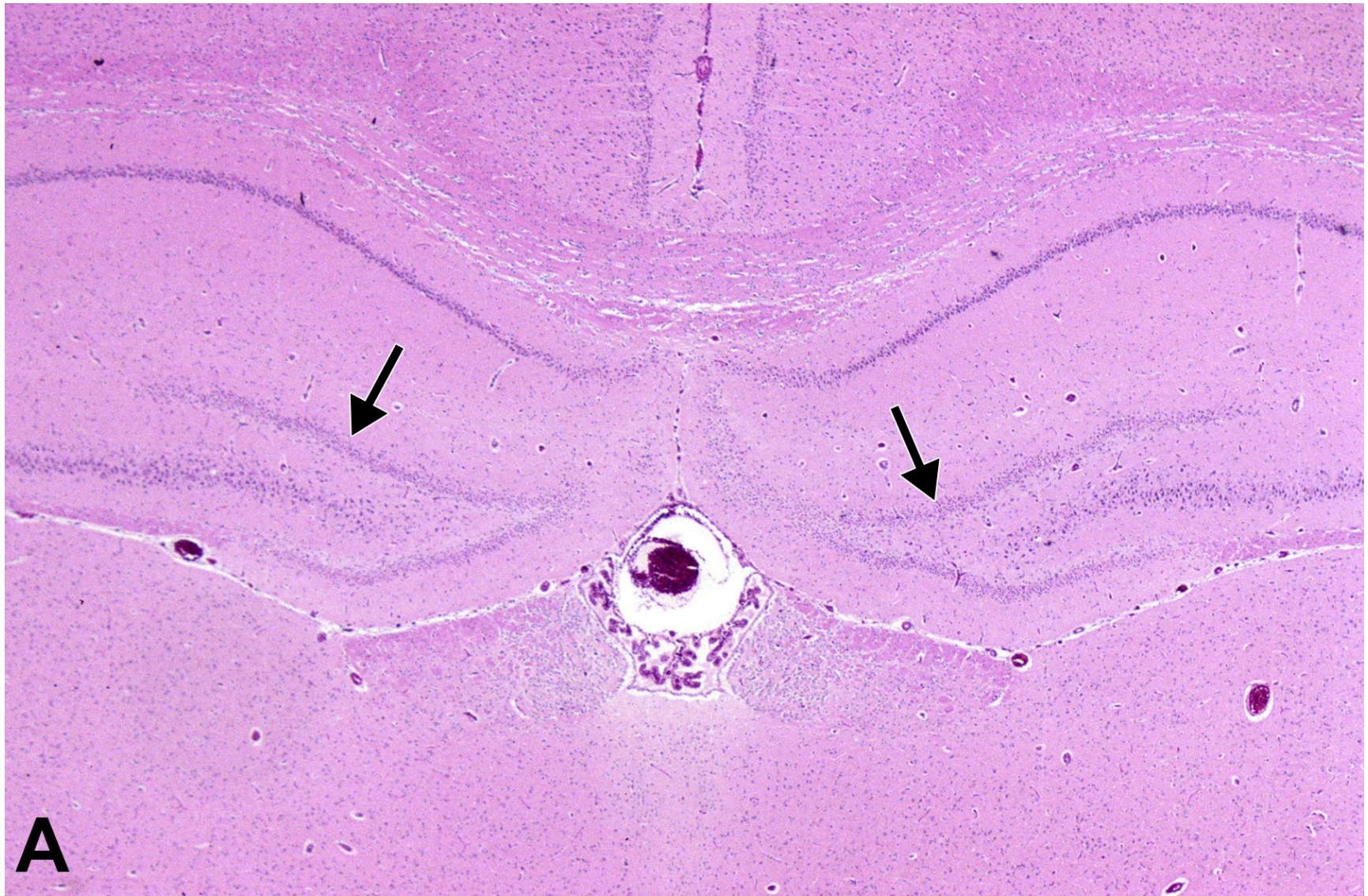


Figure 5B. Hippocampus. High magnification showing dentate gyrus neuronal necrosis. Note pyknotic neuronal nuclei (arrows). Female rat treated with 1000mg/kg benzyl acetate for 13 weeks. H&E, 13.2X.

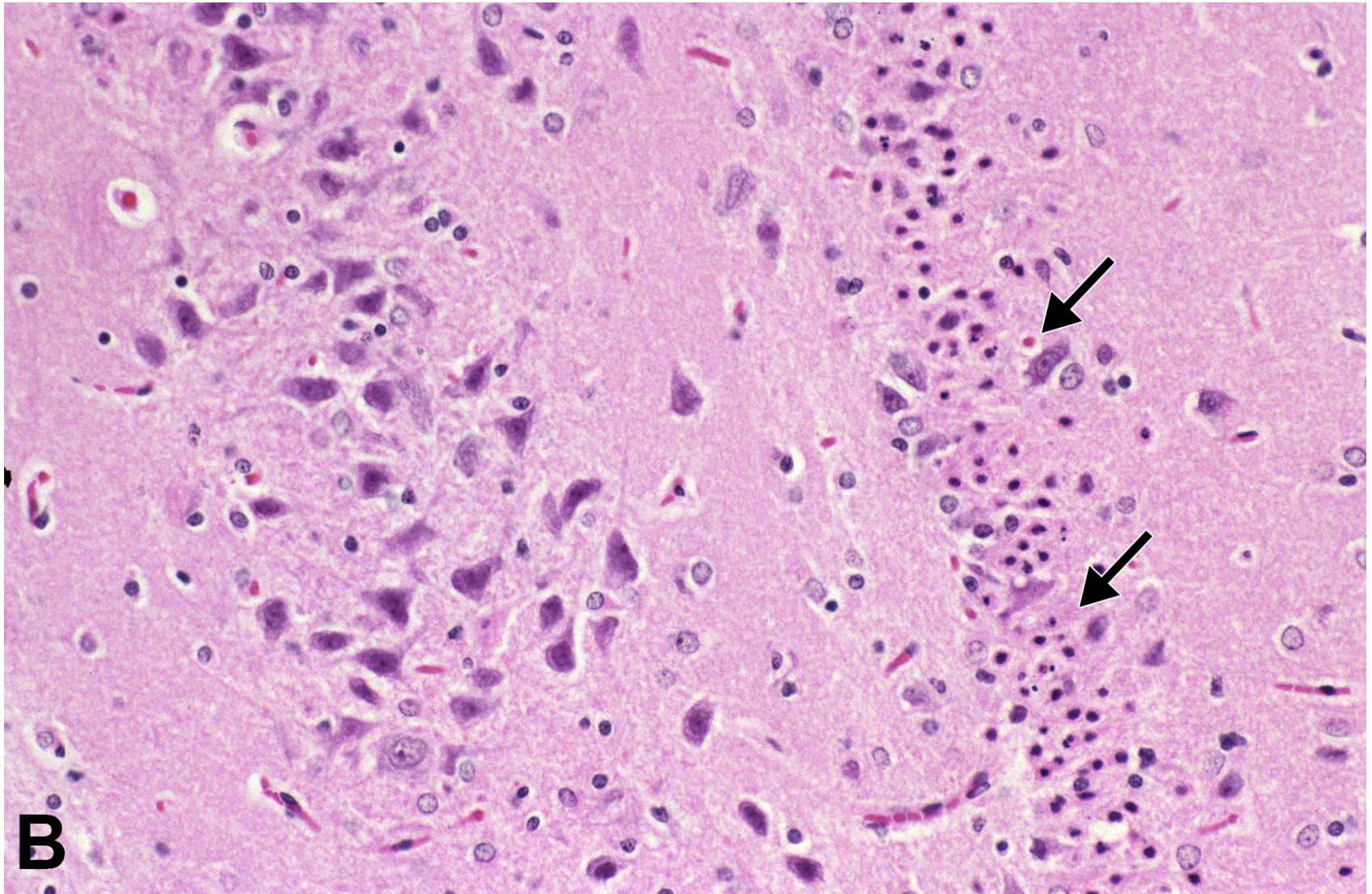
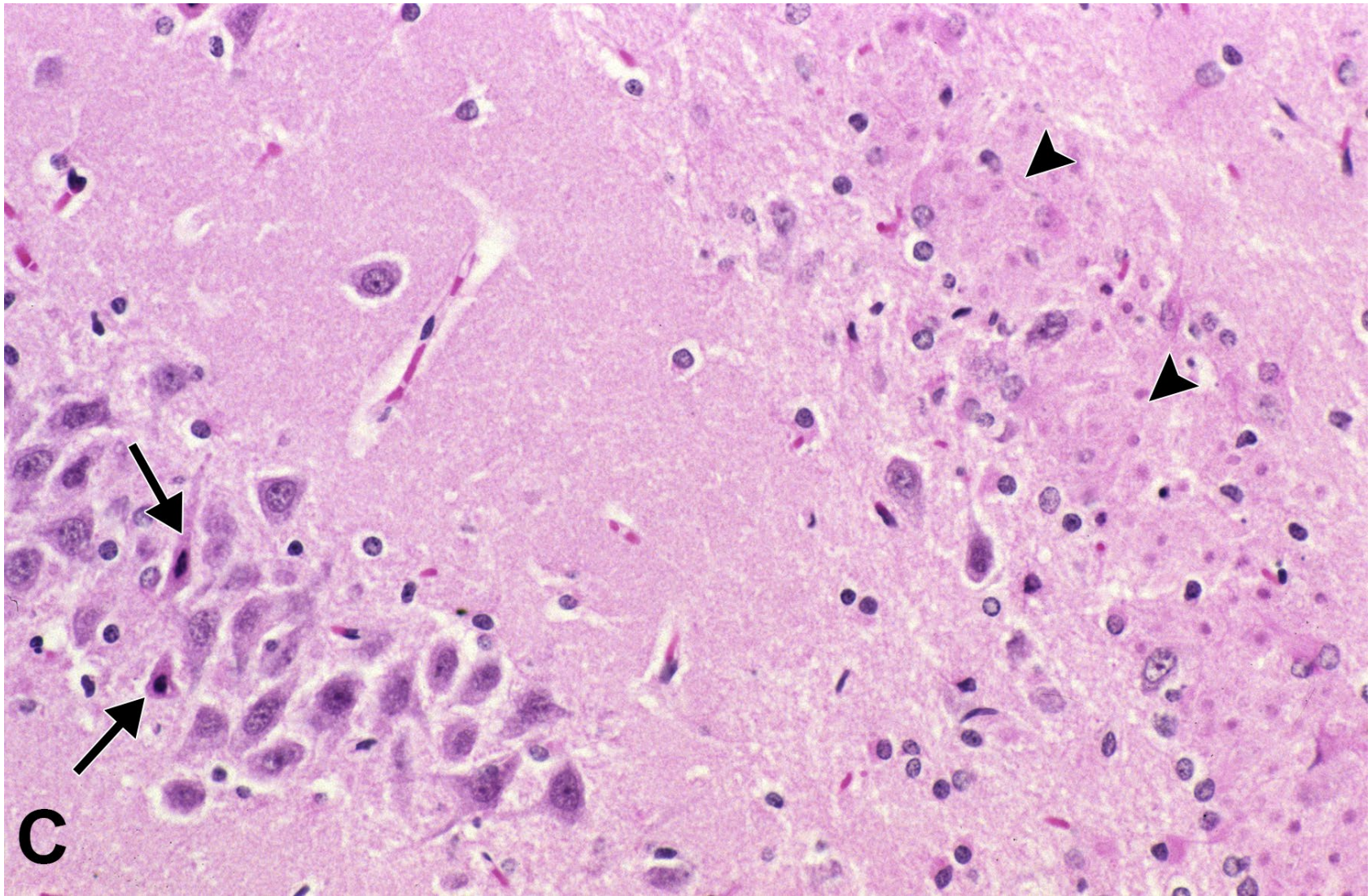


Figure 5C. Hippocampus. Dentate gyrus, complete neuronal necrosis (arrowheads). Note cornu ammonis 3 (CA3) individual neuronal necrosis (large arrows). Male rat treated with 1000 mg/kg benzyl acetate for 13 weeks. H&E, 80X.

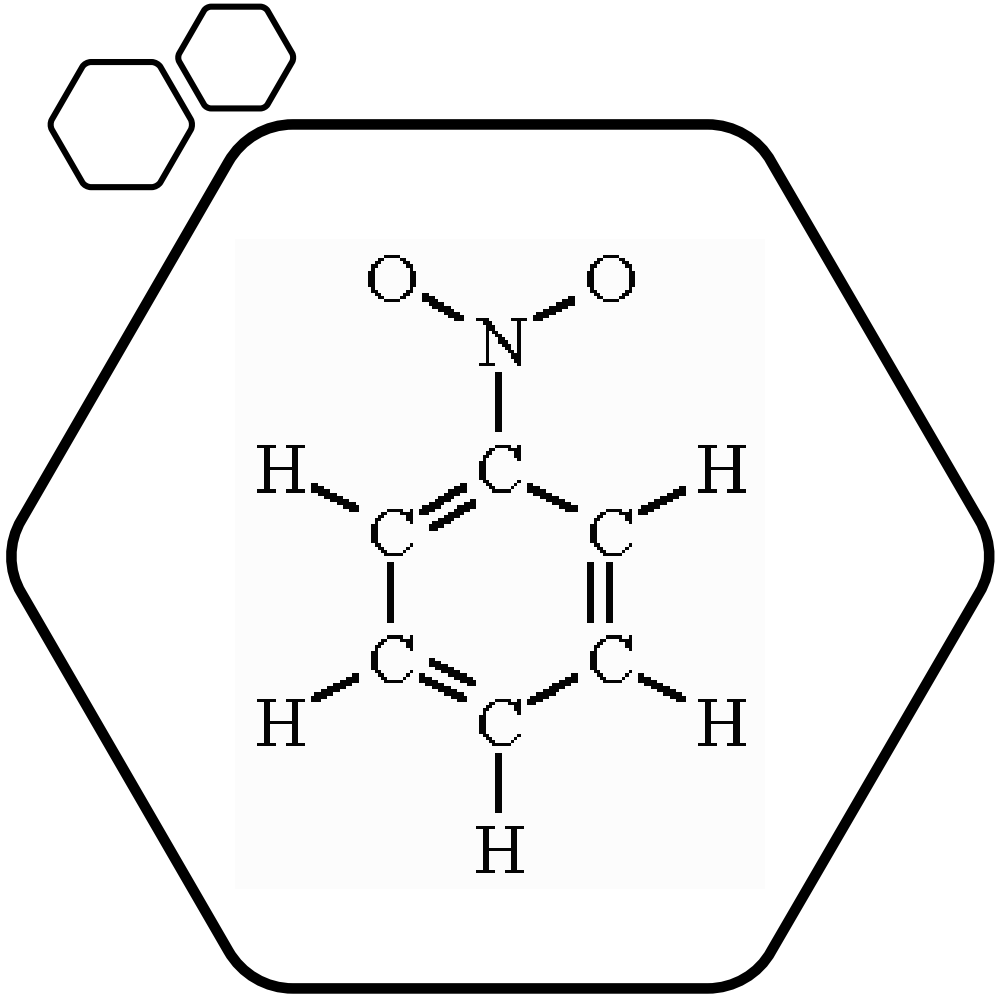


#5 Benzyl acetate Neuropathological diagnoses

- Hippocampus, dentate gyrus and cornu ammonis regions 1, 2, and 3 (CA1, 2 &3); neuron, necrosis

#6 Nitrobenzene

- Nitrobenzene is an organic compound with the chemical formula $C_6H_5NO_2$. It is a yellow oily liquid made by nitrating benzene, used in chemical synthesis and has an almond-like odor. It freezes to give greenish-yellow crystals.
- Nitrobenzene is produced on a large scale as a precursor to aniline. In the laboratory, it is occasionally used as a solvent, especially for electrophilic reagents. Nitrobenzene is also used to produce lubricating oils such as those used in motors and machinery. A small amount is used in the manufacture of dyes, drugs, pesticides, and synthetic rubber.
- Clinical signs: Ataxia and inactive prostration in rats
- Now review the following whole slide images: Figure 6-1 and Figure 6-2.



Nitrobenzene CAS No. 98-95-3: CNS Lesions

- The original histopathological report indicated the presence of clear vacuoles in the white matter of the cerebellar cortex and gray and white mater of the brain stem of F344/N rats. These vacuoles were considered potential artifacts although they were not observed in controls.
- Brain hemorrhages were seen in one control and many high-dose treated rats.
- In the review of rats exposed to nitrobenzene by gavage or dermally, there was a pattern of subacute lesions in several critical neural sites.
- Specific foci of neuronal necrosis were observed in the substantia nigra, the vestibular nuclei, the lateral anterior olivary nucleus and, occasionally, the cerebellar roof nuclei and mammillary body of the hypothalamus (Figure 6A, Figure 6B, Figure 6C, and Figure 6D).
- These lesions were characterized by loss of neurons from affected nuclei, vacuolation of the adjacent neuropil, and a variably severe gliosis.
- This gliosis was comprised of microglia, rare macrophages and, sometimes, anisomorphic astrogliosis (consisting of astrocytic responses in which the underlying normal brain structure was significantly distorted.)

Nitrobenzene CAS No. 98-95-3: CNS Lesions

- Lesions in these brain regions would be expected to compromise extrapyramidal motor activity (substantia nigra), perhaps causing immobility and tremors, impaired motor coordination (vestibular and cerebellar roof nuclei) and disturbed metabolic/hormonal functions (mammillary body) in affected animals.
- The nature of the neural lesions suggested unique neuronal specificity for this compound. Even the affected regions with large lesions were not totally necrotic but had a selective death of individual neurons in a nucleus with survival of adjacent neuropil. The relative lack of macrophages in the lesions suggested a slow involution of neurons rather than massive acute necrosis.

Figure 6A. Hypothalamus. Mammillary body. Unique bilateral focal neuronal loss with vacuolation (arrows). Female rat treated with 150 mg/kg nitrobenzene for 13 weeks, H&E, 25X.

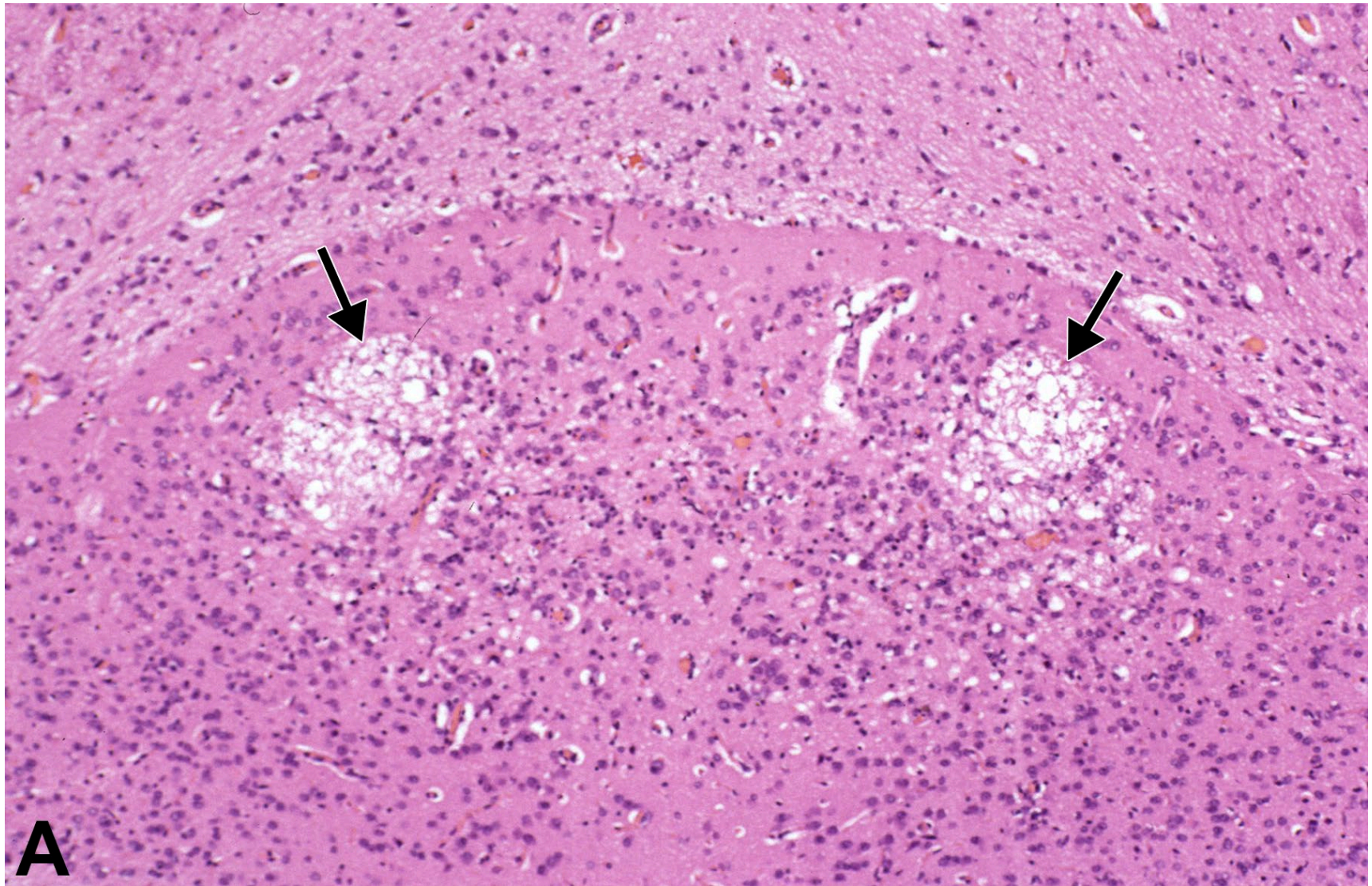
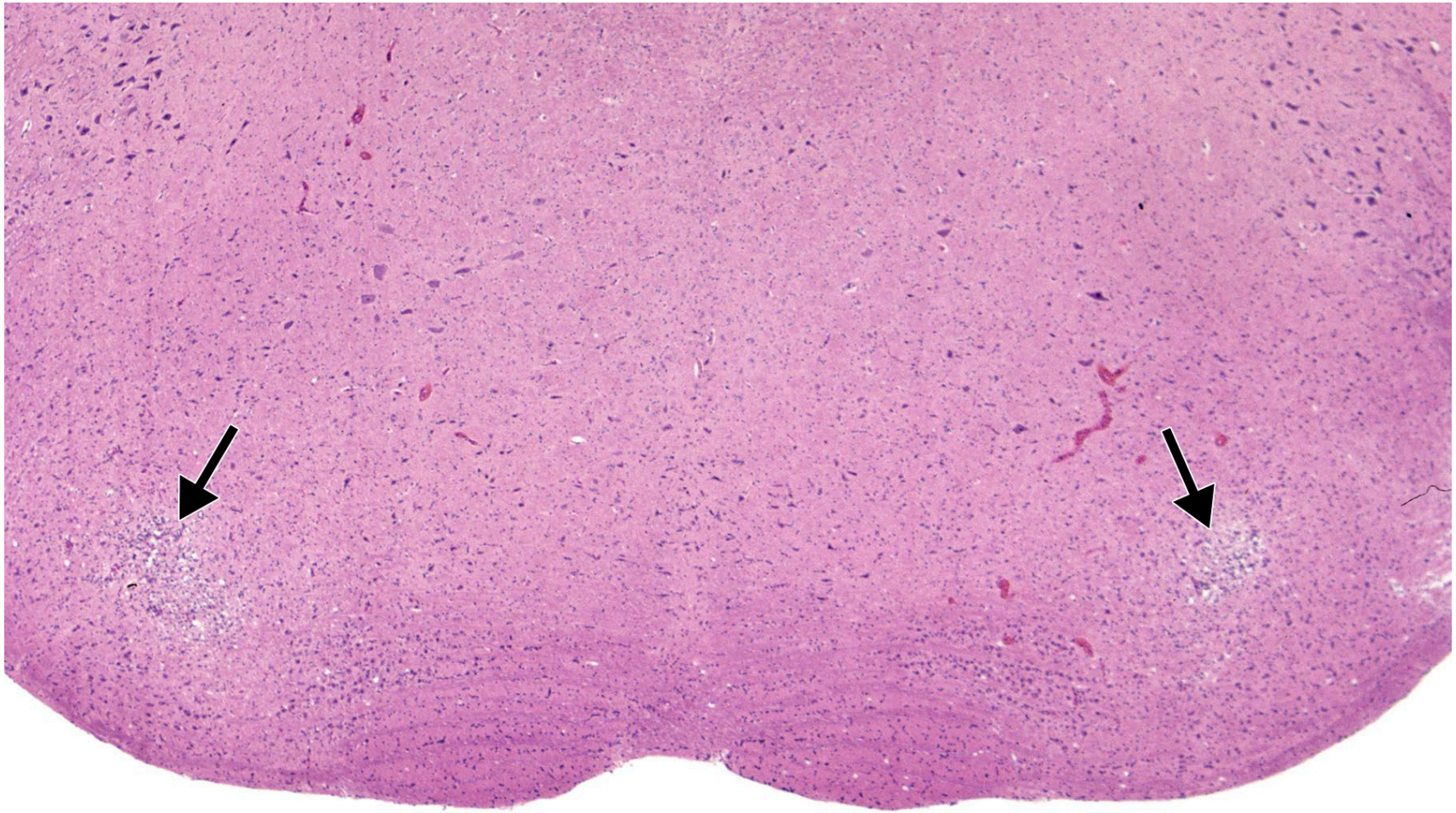


Figure 6B. Medulla. Bilateral anterior olivary nuclear necrosis (arrows) with reactive gliosis. Male rat treated with 150 mg/kg nitrobenzene for 13 weeks H&E, 6.6X.



B

Figure 6C. Note the pallor in the vestibular nuclear region (arrowheads). Female rat treated with 150 mg/kg nitrobenzene for 13 weeks, H&E, 5X.

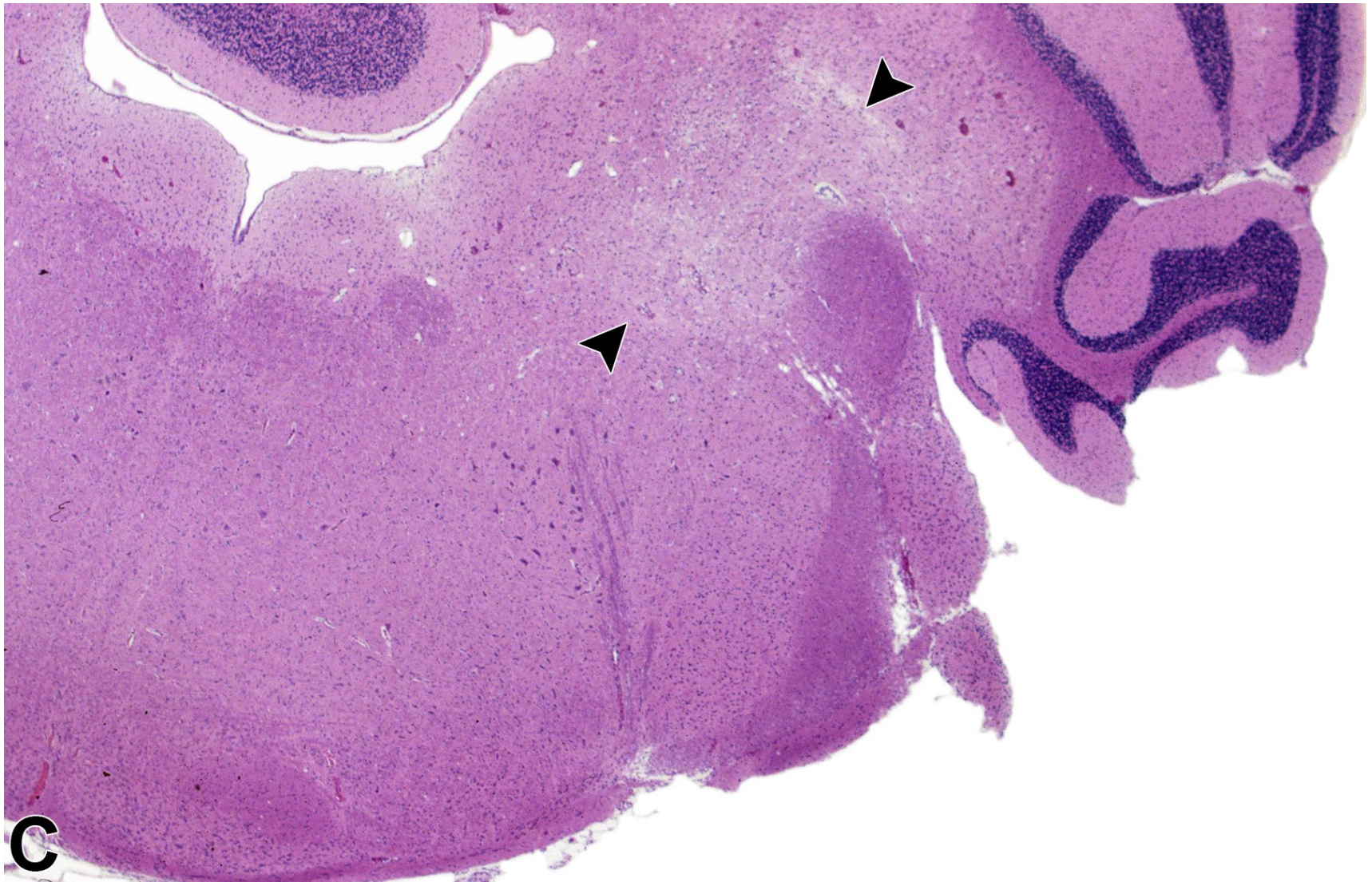
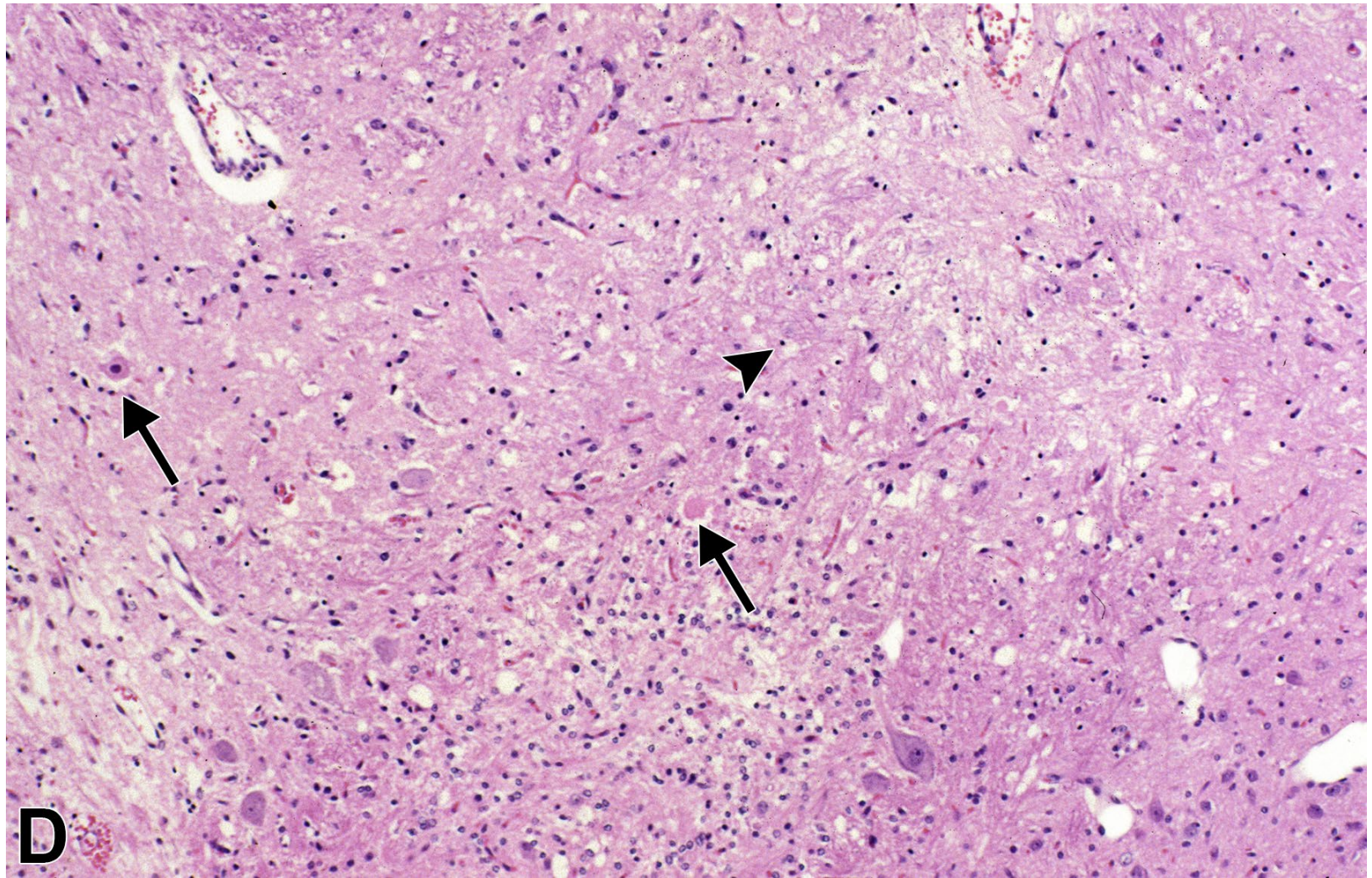


Figure 6D. High magnification of 6C. Note the necrotic neuron with pyknotic nucleus (left arrow) and one spheroid (right arrow). Neuropil vacuolation and pyknotic glial cell nuclei (arrowhead) are visible throughout the microscopic field accounting for the pallor in the low magnification image. Female rat treated with 150 mg/kg nitrobenzene for 13 weeks H&E, 25X.

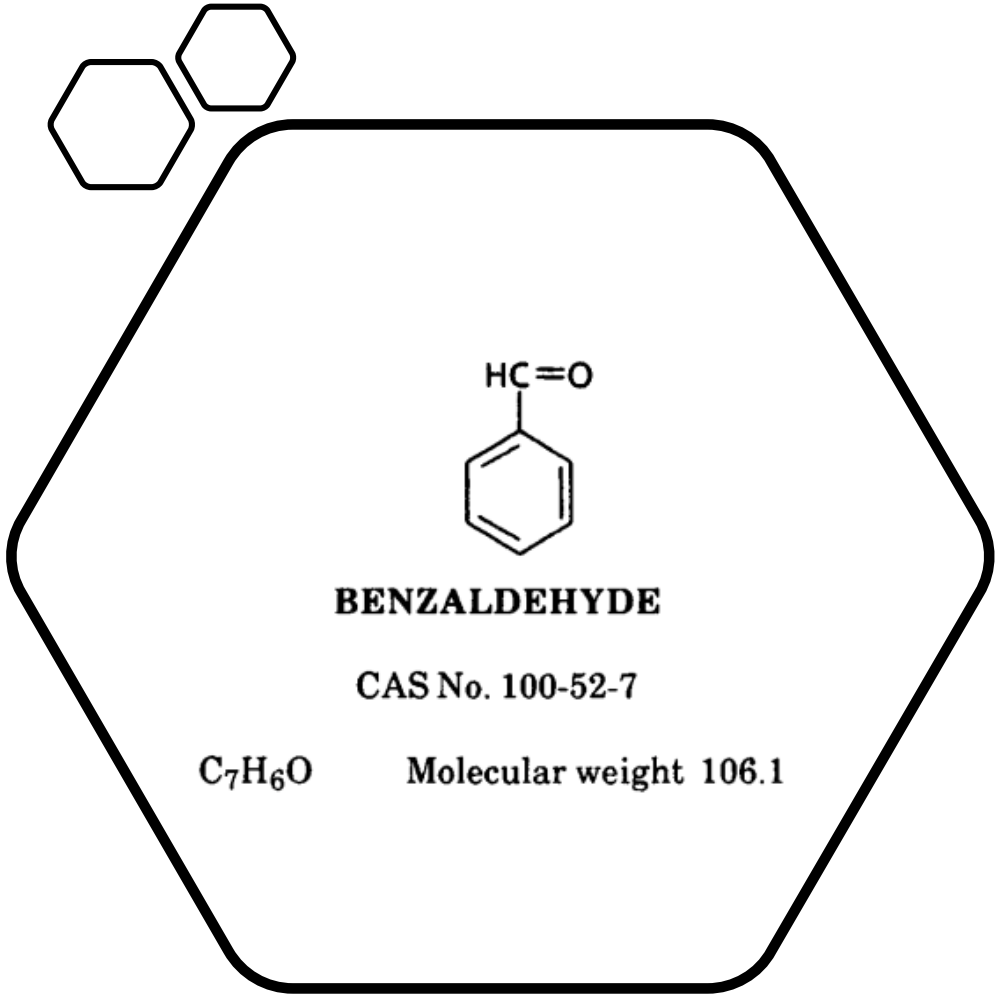


#6 Nitrobenzene Neuropathological diagnosis

- Substantia nigra, vestibular nuclei, lateral anterior olivary nucleus, cerebellar roof nuclei, hypothalamic mammillary body; neuron, necrosis.

#7 Benzaldehyde

- (C_6H_5CHO) is an organic compound consisting of a benzene ring with a formyl substituent. It is the simplest aromatic aldehyde and one of the most industrially useful.
- It is a colorless liquid with a characteristic almond-like odor. The primary component of bitter almond oil, benzaldehyde can be extracted from other natural sources. Synthetic benzaldehyde is the flavoring agent in imitation almond extract, which is used to flavor cakes and other baked goods.
- Clinical signs: Hyperactivity, trembling, & paralysis in rats
- Now review the following whole slide images: Figure 7-1, Figure 7-2, Figure 7-3, Figure 7-4, and Figure 7-5.



Benzaldehyde National Toxicology Program, (1990): CNS Lesions

- Brain lesions in the male and female rats consisted of hippocampal and cerebellar cortical necrosis.
- In the cerebellar cortex, neuronal necrosis primarily affected the internal granule cells. Moderate Purkinje cell necrosis was evident when the internal granular cell lesions were moderate to marked in severity.
- The cerebellar lesions were accompanied by mineralization in the more extensively necrotic zones and laminated calcospherites were evident in these foci.
- In the white matter of the cerebellum, vacuolation was probably related to loss of Purkinje cell axons since some vacuoles contained swollen axonal bodies but more commonly wisps of degenerate material.
- In the hippocampus, the dentate gyrus and cornu ammonis regions 1 and 3 (CA1 and CA3) regions were primarily affected. In the dentate gyrus, there was generally complete necrosis of the granule cell neurons accompanied by variably severe reactive gemistocytic astrocytosis (Figure 7A and Figure 7B).
- In CA1 & CA3 regions, the neuronal necrosis was less complete but was generally severe and extensive; cornu ammonis region 2 (CA2) was not affected.

Benzaldehyde National Toxicology Program, (1990): CNS Lesions

- In two sections where the amygdaloid nucleus was present, necrosis of this important behavioral nucleus was evident (Figure 7C) and, in the one case where both sides of the amygdaloid were present, the lesion was bilateral.
- In females, the cerebellar brain lesions were minimal to moderate in severity. However, the hippocampal lesions were as severe as those in males and were accompanied by variable degrees of gemistocytic astrocytosis.
- Cerebellar cortical lesions were not accompanied by mineralization as in males suggesting that mineralization was a function of severity and probably duration of internal granule cell necrosis.
- Amygdaloid lesions were not seen in females.
- This review confirmed the presence of cerebellar cortical and hippocampal lesions observed in the original histopathology evaluation and extended the descriptive specificity of the lesions.
- The presence of mineralization of cerebellar cortical lesions in males indicated the severity of the compound-related brain effect in this sex as well as the chronicity of the process during the 13-week trial period.

Figure 7A. Hippocampus. Neuronal necrosis and loss, dentate gyrus, cornu ammonis 1 and 3 (CA1 and CA3). Note cornu ammonis 2 (CA2) is relatively spared (perimeter bars). Male rat treated with 800 mg/kg benzaldehyde for 13 weeks. H&E, 10X.

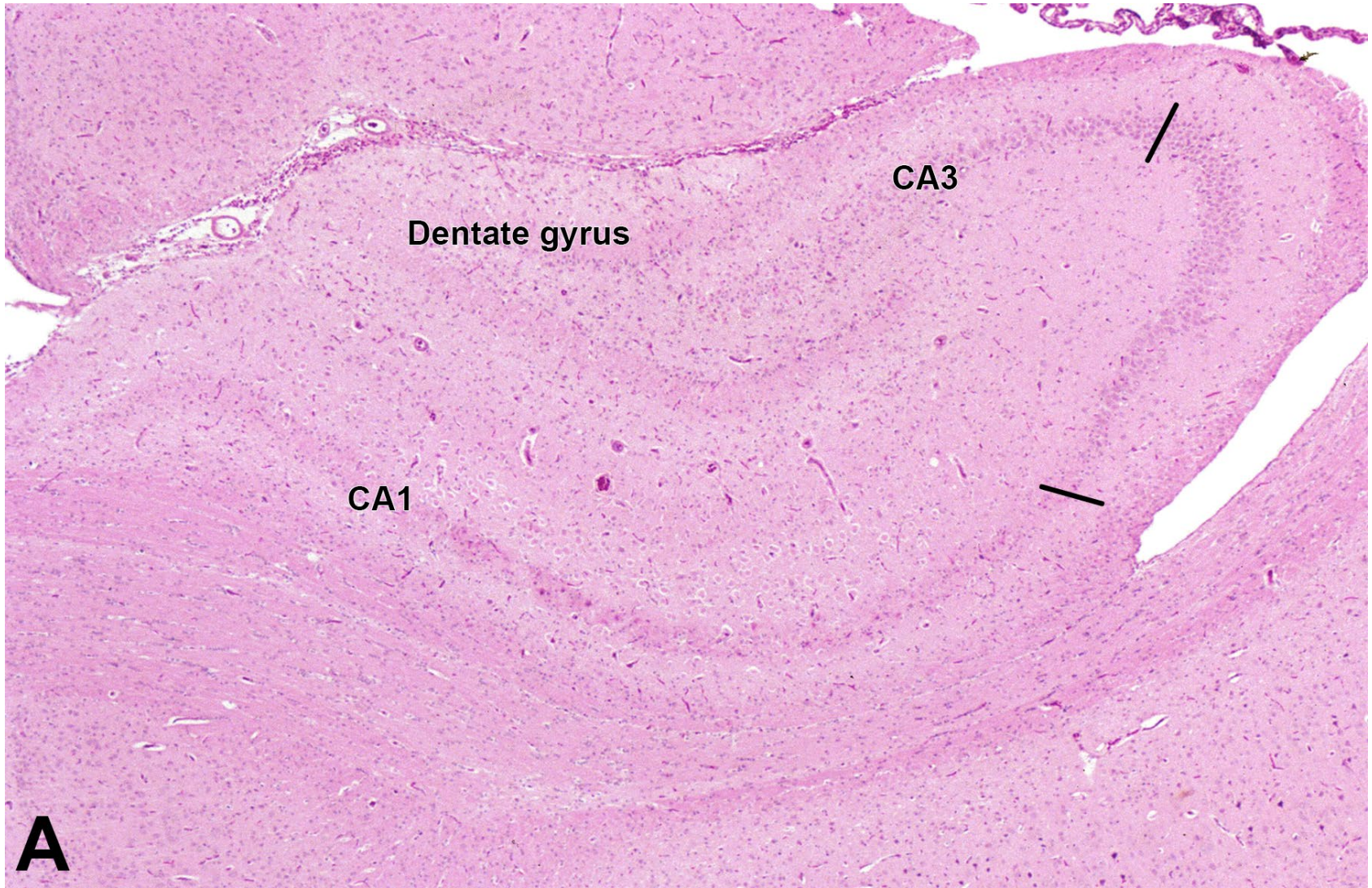


Figure 7B. Hippocampus. Dentate gyrus neuronal necrosis with reactive gemistocytic astrocytes (arrows). Male rat treated with 800 mg/kg benzaldehyde for 13 weeks. H&E, 66X.

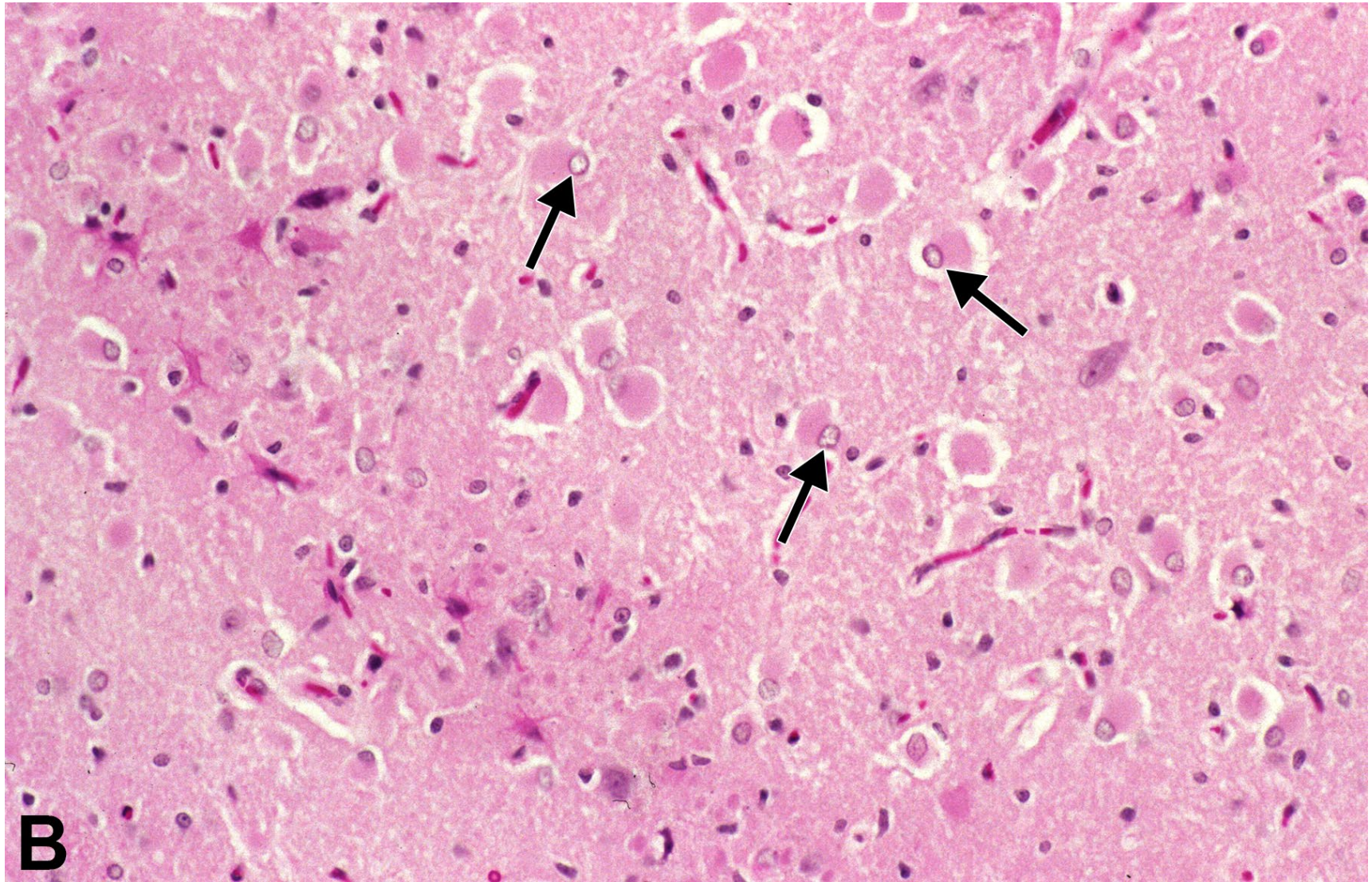
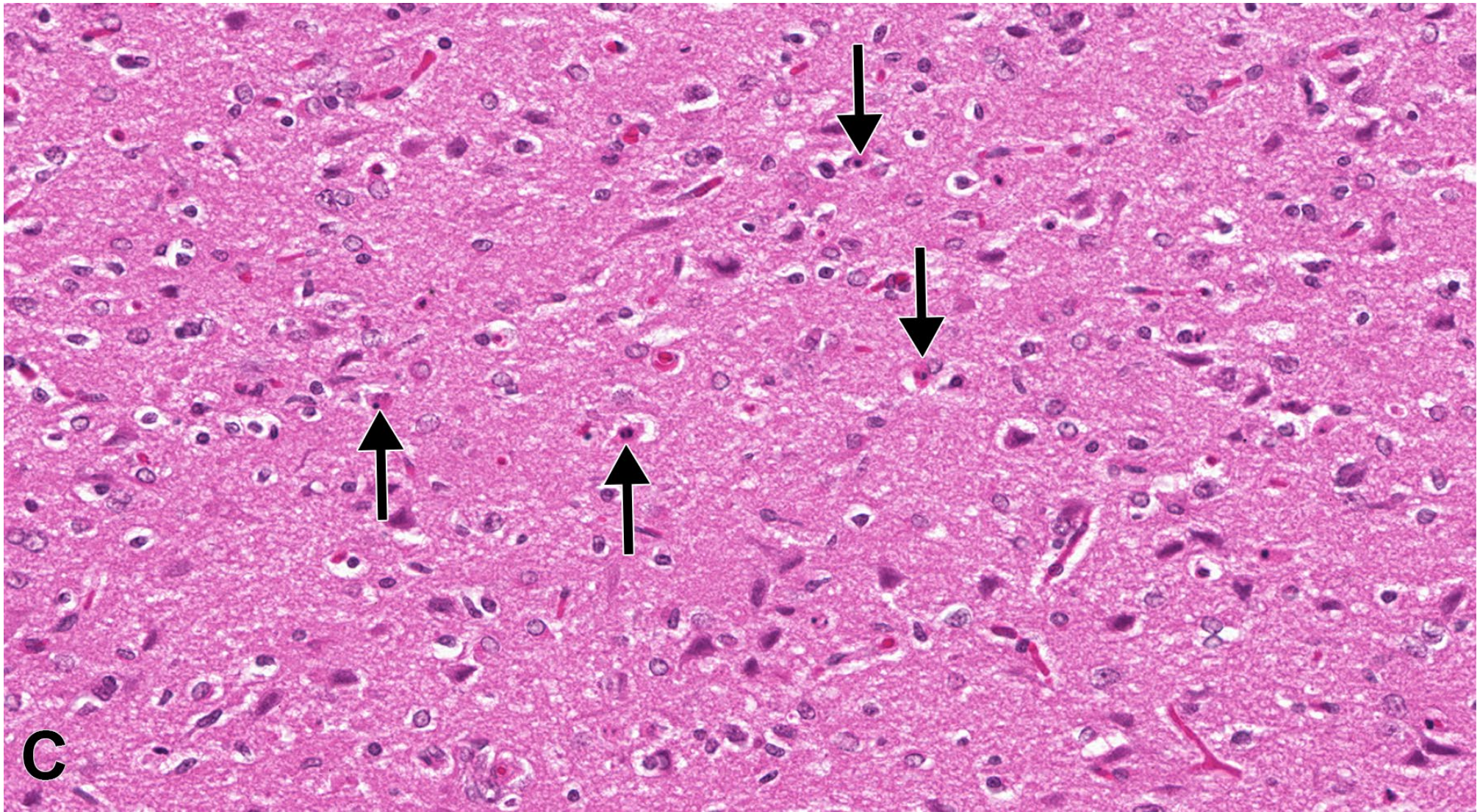


Figure 7C. Cerebrum, amygdaloid nucleus. Note scattered neuronal necrosis (arrows). Male rat treated with 800 mg/kg benzaldehyde for 13 weeks. H&E, 34.4X.

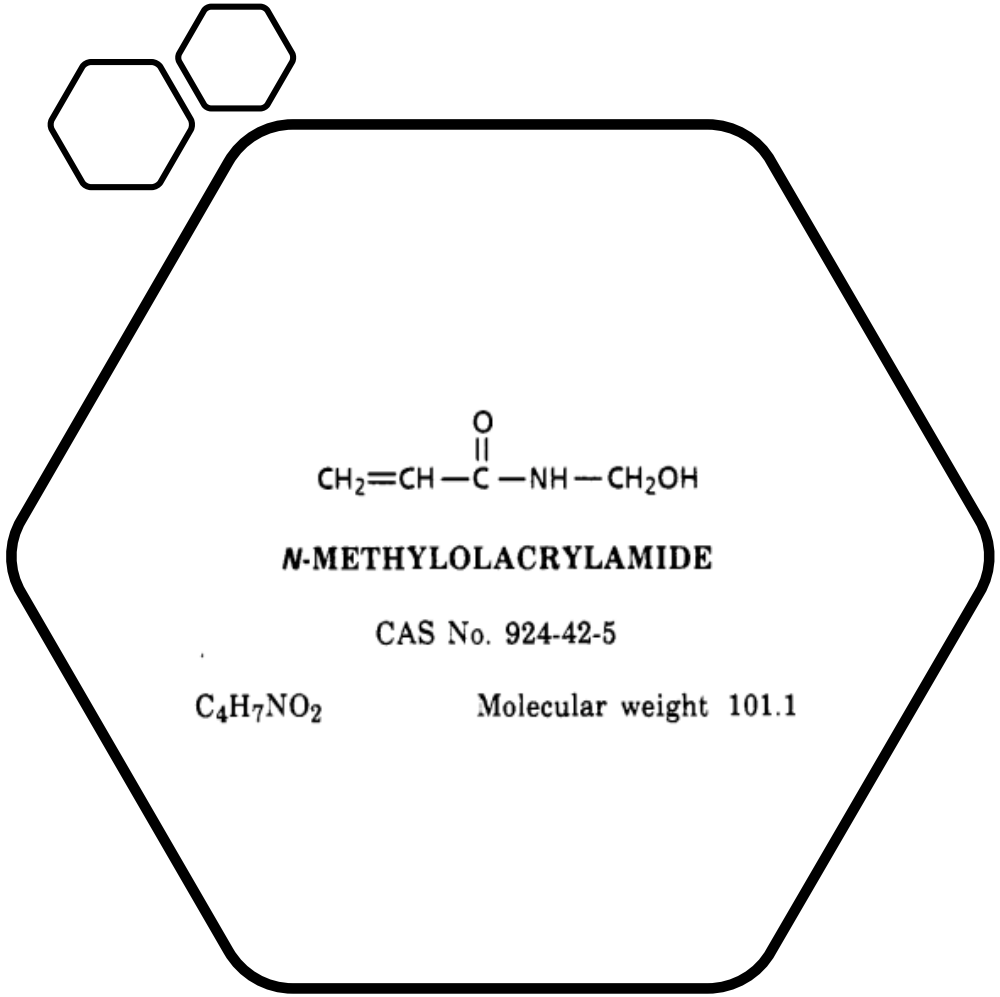


#7 Benzaldehyde Neuropathological diagnoses

- Hippocampus dentate gyrus and cornu ammonis 1 and 3 (CA1 and CA3), amygdaloid nucleus, cerebellar cortex (internal granule cell and Purkinje cell); neuron, necrosis.

#8 N-Methylolacrylamide

- N-Methylolacrylamide is a cross-linking agent used in adhesives, binders for paper, crease-resistant textiles, resins, latex film, and sizing agents.
- Clinical signs: Irritability, inactivity, ataxia, & paralysis in rats
- Now review the following whole slide images: Figure 8-1, Figure 8-2, Figure 8-3a, Figure 8-3b, Figure 8-3c, Figure 8-3d, and Figure 8-4.



N-Methylolacrylamide National Toxicology Program, (1989): CNS/PNS Lesions

- Behavioral tests in F-344 rats conducted during the sixth and thirteenth weeks on study indicated a reduction in grip strength, decrease in startle response and an increase in landing foot-spread.
- In the original review, axon fiber and myelin sheath degeneration were observed in the cerebellum, medulla, and spinal cord. Also reported were extensive degenerative lesions in both tibial and plantar nerves from most animals of both sexes in the treated groups.
- The most severe lesions were described as diffuse and profound degeneration and loss of nerve fibers, swelling and fragmentation of axis cylinders, accumulation of lamellar bodies and dense bodies.
- Also described were myelin retraction at the nodes of Ranvier, myelin blebbing, and thinning and loss of myelin sheaths.

N-Methylolacrylamide National Toxicology Program, (1989): CNS/PNS Lesions

- In the retrospective review, the cerebellar lesions were further characterized as internal granule cell lesions in the high-dose animals of both sexes, occasionally accompanied by mineralization of necrotic cells and processes (see whole slide image Figure 8-1).
- In addition, acute eosinophilic degeneration was observed in Purkinje cells; also frequently referred to as “ischemic” or metabolic arrest changes, characterized by contracted eosinophilic cytoplasm and shrunken fragmented basophilic nuclei (see whole slide image Figure 8-2)
- Also present were various stages of neuronal chromatolysis and necrosis in the medulla (Figure 8A).
- In the spinal cord, the predominant lesion was axonopathy of the dorsomedial region of the dorsal funiculus (Figure 8B; also see whole slide image Figure 8-2). This corresponds to the gracile proprioceptive pathway in a section that was taken from the posterior cervical region in the area of the brachial intumescence.
- Only occasional swollen axons were visible; however, the neuropil was condensed and astroglial cell hyperplasia and hypertrophy indicated that fiber loss and cellular reaction to the process had occurred (see whole slide image Figure 8-2).

N-Methylolacrylamide National Toxicology Program, (1989): CNS/PNS Lesions

- Tibial and plantar nerve changes observed were similar in most instances to those mentioned in the original review.
- The most prominent and notable changes, in severe cases, were those of fiber loss, Schwann cell nuclear hypertrophy, plus thin myelin sheaths indicating some attempt at fiber regeneration and remyelination (Figure 8C and Figure 8D; also see whole slide images Figure 8-3a, Figure 8-3b, Figure 8-3c, Figure 8-3d, and Figure 8-4).
- Additionally, myelin ovoid bodies were apparent, with many laminated (also see whole slide images Figure 8-3c, Figure 8-3d, and Figure 8-4). This indicated the presence of myelin degeneration, and the bodies were present in digestion chambers formed by Schwann cells and macrophages.

Figure 8A. Medulla. Various stages of chromatolysis (arrows) and necrosis (arrowhead) of fifth cranial nerve motor neurons. Female rat treated with 200 mg/kg methylolacrylamide for 13 weeks. H&E, 100X.

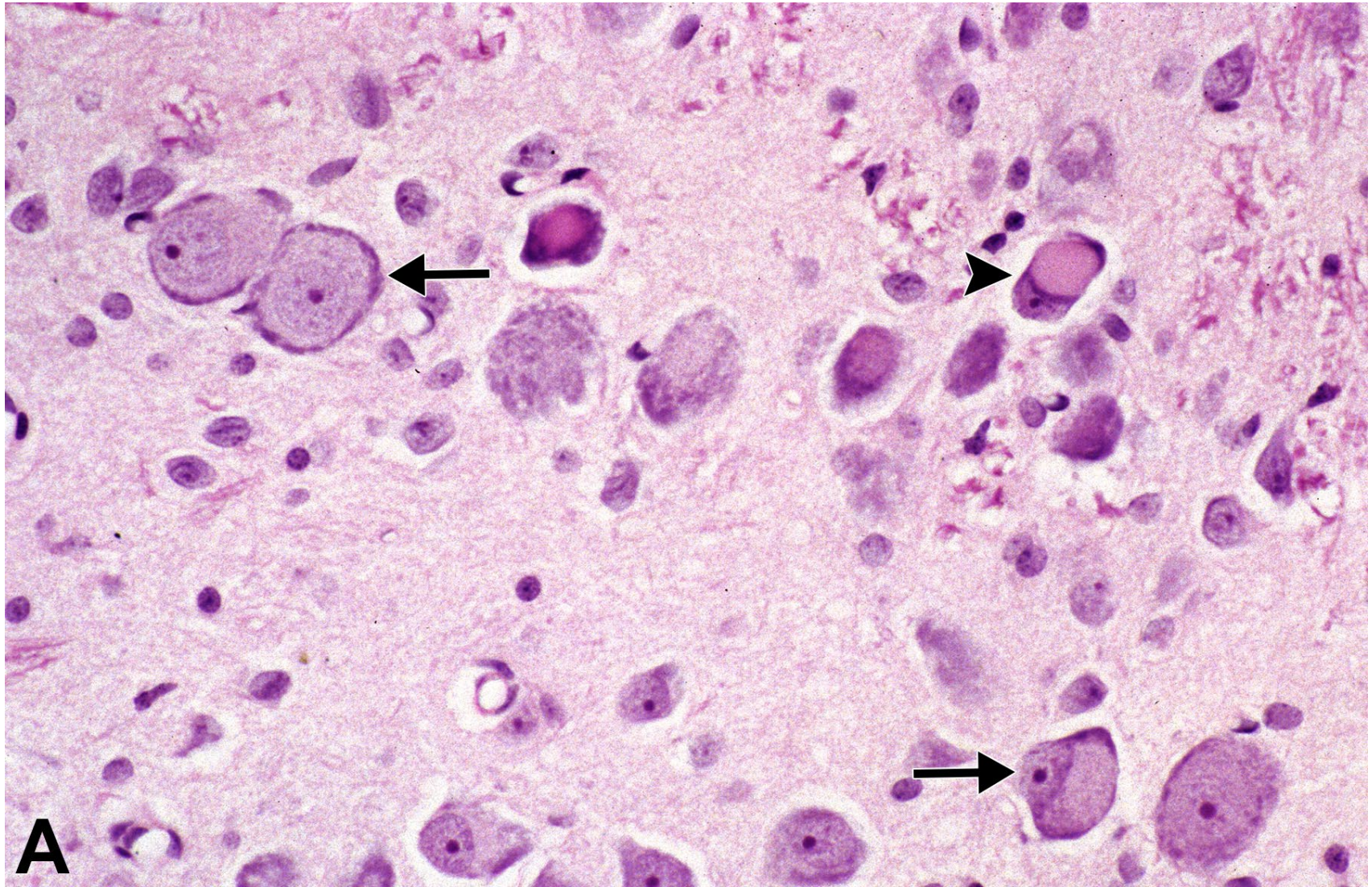


Figure 8B. Spinal cord. Dorsolateral funiculus axonopathy characterized by eosinophilic axonal spheroids (arrows). Male rat treated with 100 mg/kg methylolacrylamide for 13 weeks. H&E, 80X.

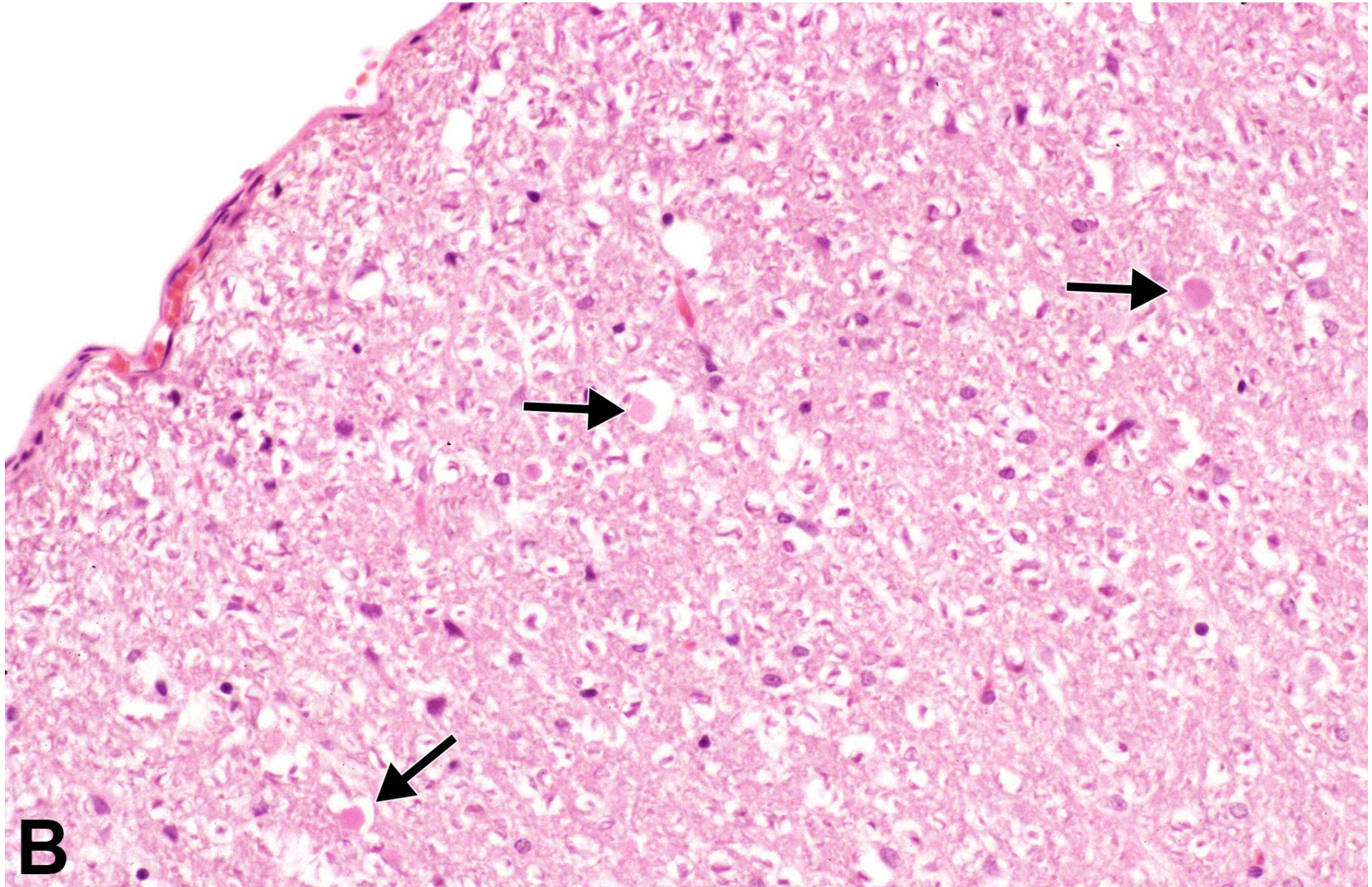


Figure 8C. Tibial nerve from control female rat. Toluidine blue stain, 100X.

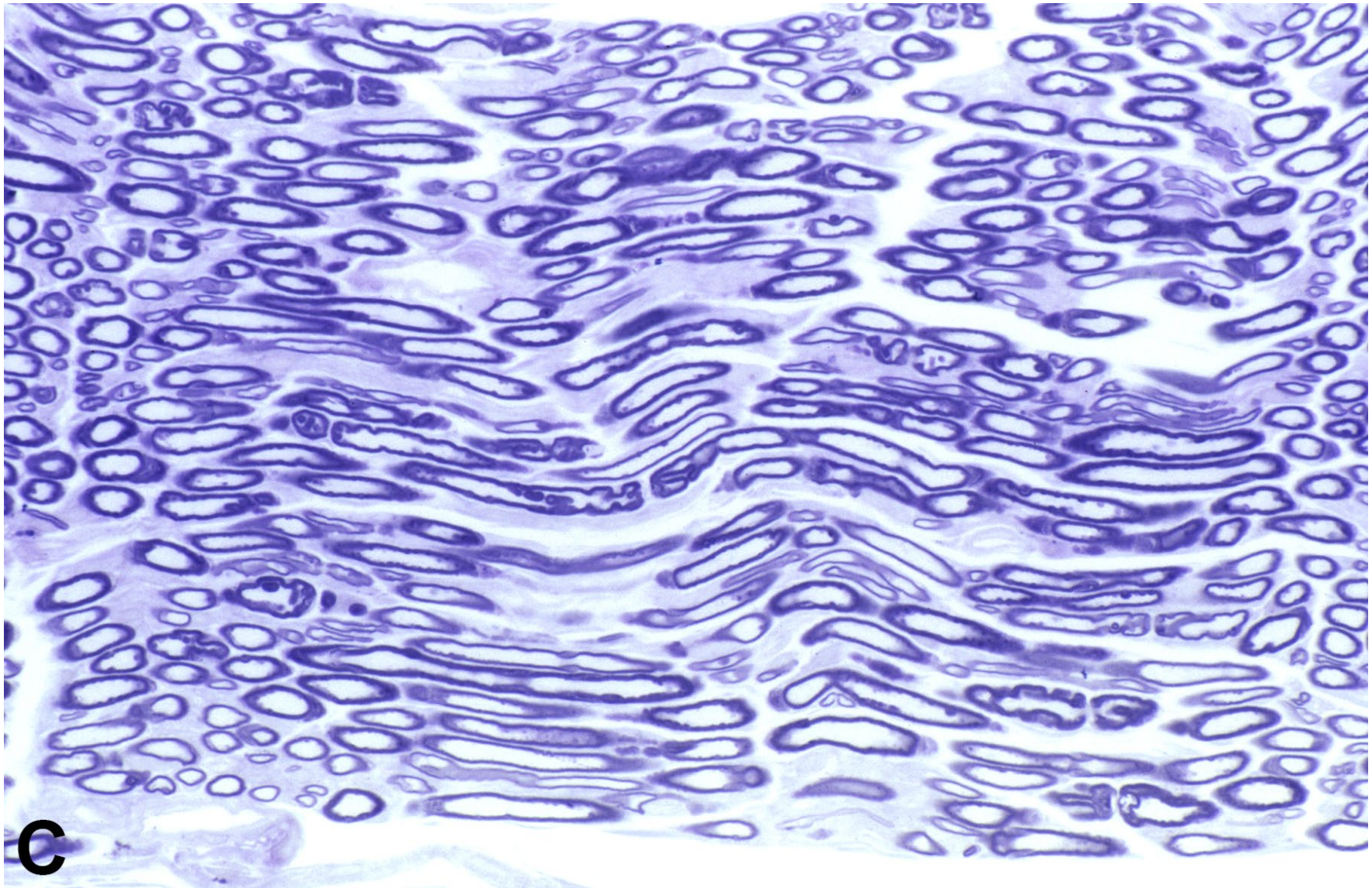
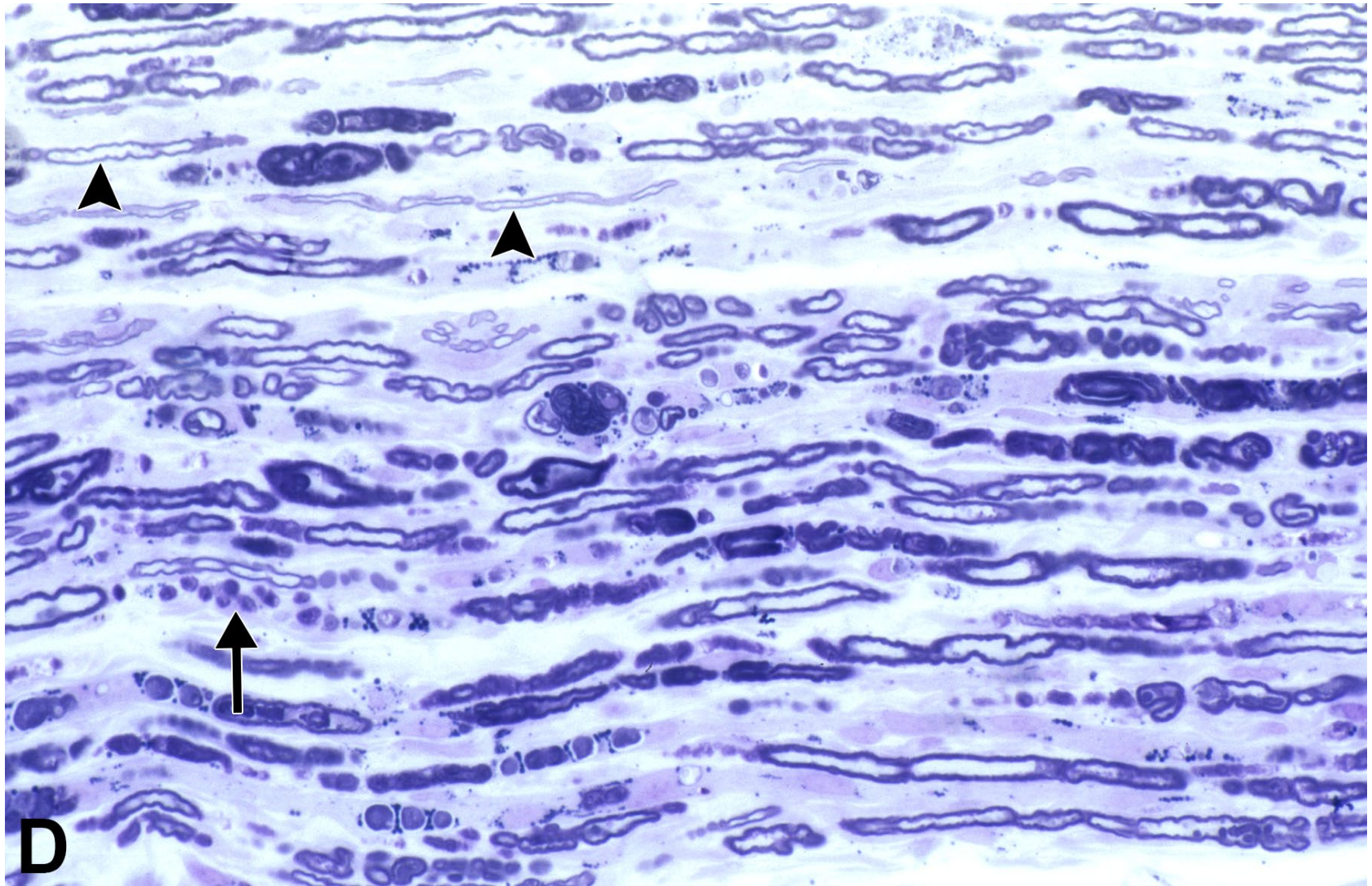


Figure 8D. Tibial nerve. Compared to the control (figure 8C) there is fiber loss accompanied by myelin ovoids (arrow) and thin myelin sheaths (arrowheads). Female rat treated with 100 mg/kg methylolacrylamide for 13 weeks. Toluidine blue stain, 100X.

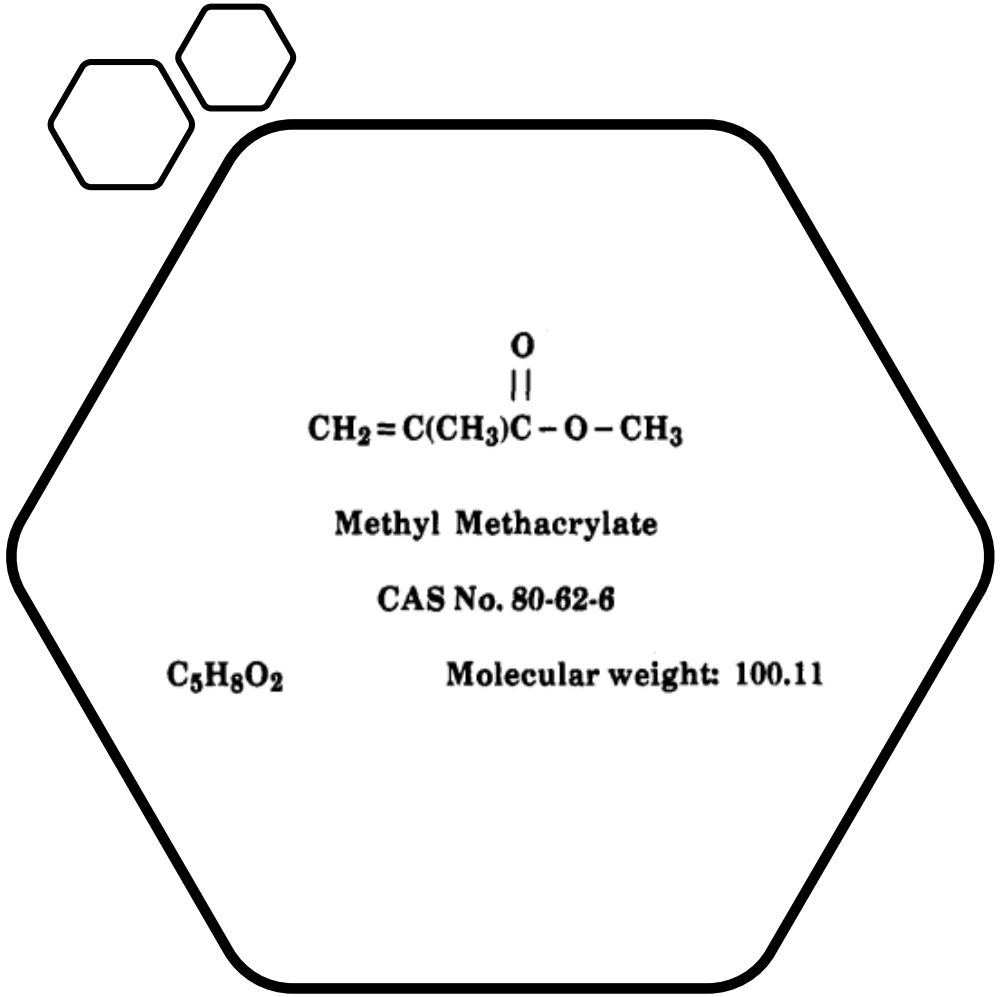


#8 N-Methylolacrylamide Neuropathological diagnoses

- Cerebellum, medulla, and spinal cord, tibial and plantar nerves; neuron, axonopathy.
- Cerebellar internal granule cell, Purkinje cell; neuron, necrosis.
- Medulla 5th cranial nerve nucleus; neuron, necrosis

#9 Methyl Methacrylate

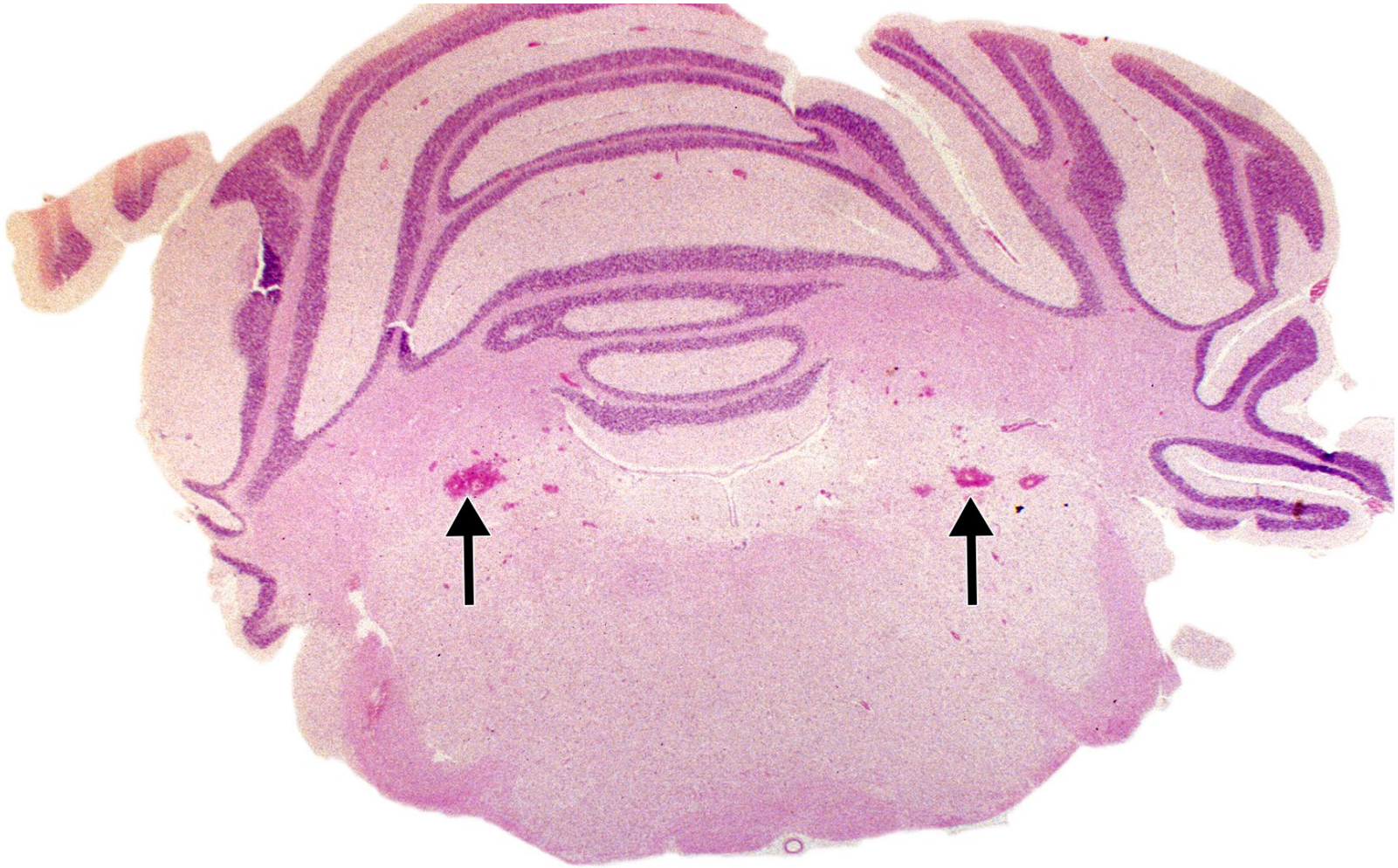
- Methyl Methacrylate is an organic compound. It is a colorless liquid and is a monomer produced on a large scale to produce poly(methyl methacrylate) (PMMA). This is used in many forms of common plastic objects.
- Clinical signs: Listlessness & incoordination in rats
- Now review the following whole slide images: Figure 9-1a, Figure 9-1b, Figure 9-2, Figure 9-3a, and Figure 9-3b.



Methyl Methacrylate National Toxicology Program (NTP) (1986 B): CNS Lesions

- Microscopically, brain congestion was recorded in male and female rats of the high-dose group. Hemorrhage, malacia, and gliosis of the cerebellum were observed in high dose females.
- In the retrospective review, only female animals in the mid-high dose groups were available for examination and all had lesions in the cerebellar roof nuclei and/or vestibular nuclei if the areas had been sectioned.
- Lesions in the mid-high dose group females were generally those of acute bilateral hemorrhage with some neutrophil infiltration and acute swelling or lysis of neurons in the affected areas (Figure 9A).
- In occasional examples, the affected areas had deposits of presumptive hemosiderin pigment in macrophages suggesting old hemorrhage (Figure 9B).
- In the mid-high dose group females, the lesions were more chronic with loss of neurons and vacuolation of neuropil accompanied by astrogliosis (Figure 9C) and often the presence of macrophages (gitter cells).
- The neuronal loss and gliosis identified in the cerebellar roof nuclei and vestibular nuclei correlated with the signs of incoordination recorded clinically in rats.

Figure 9A. Medulla. Note bilateral vestibular nucleus hemorrhage (arrows). Female rat exposed to the mid-high dose of 2000 ppm methylmethacrylate by inhalation for 13 weeks. H&E, 2.5X.



A

Figure 9B. Cerebellar roof nuclei. Note hemosiderin-like pigment in macrophages, suggesting prior hemorrhage in an area of gliosis. Female rat exposed to 2000 ppm methylmethacrylate by inhalation for 13 weeks. H&E, 66X.

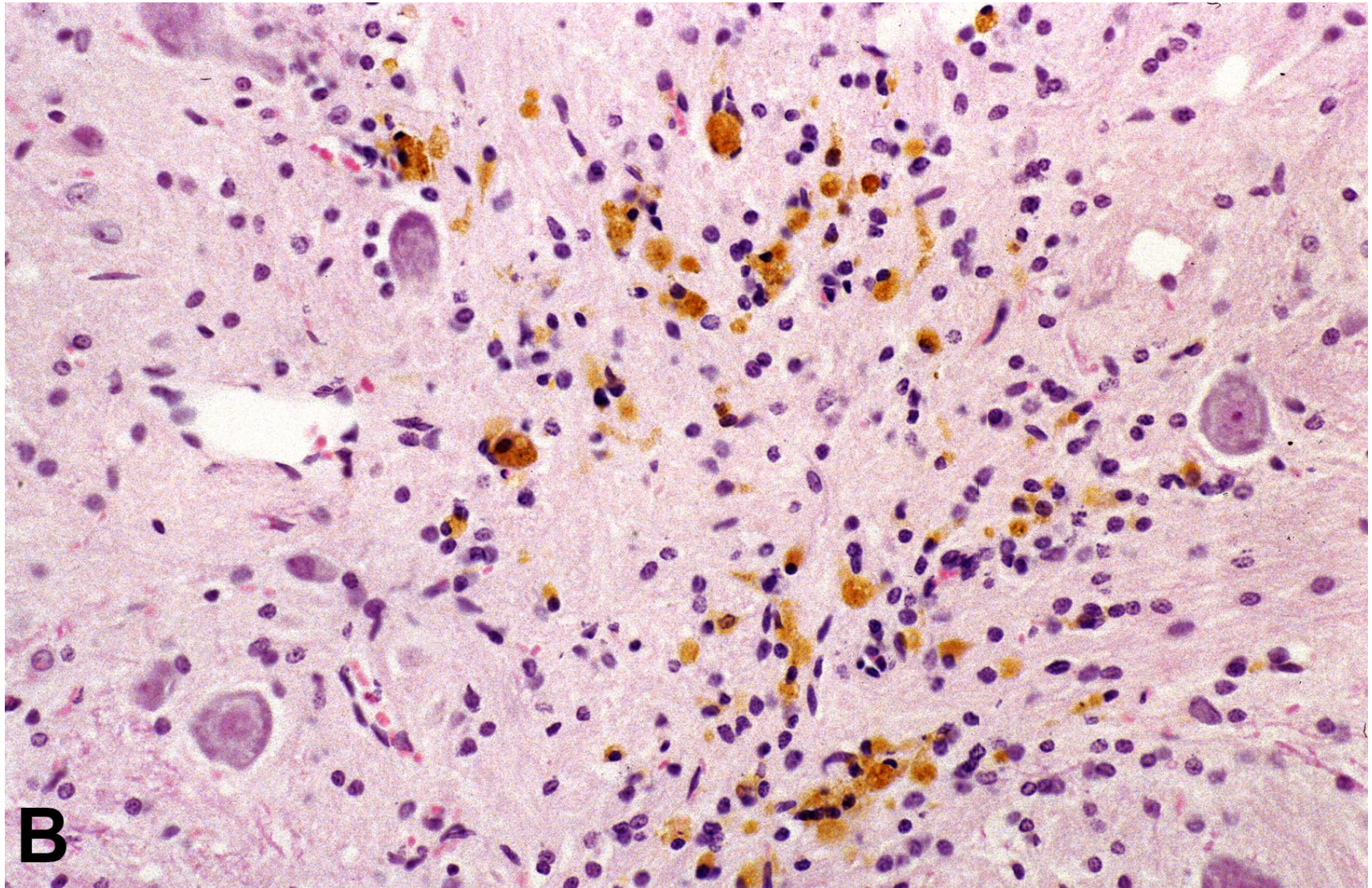
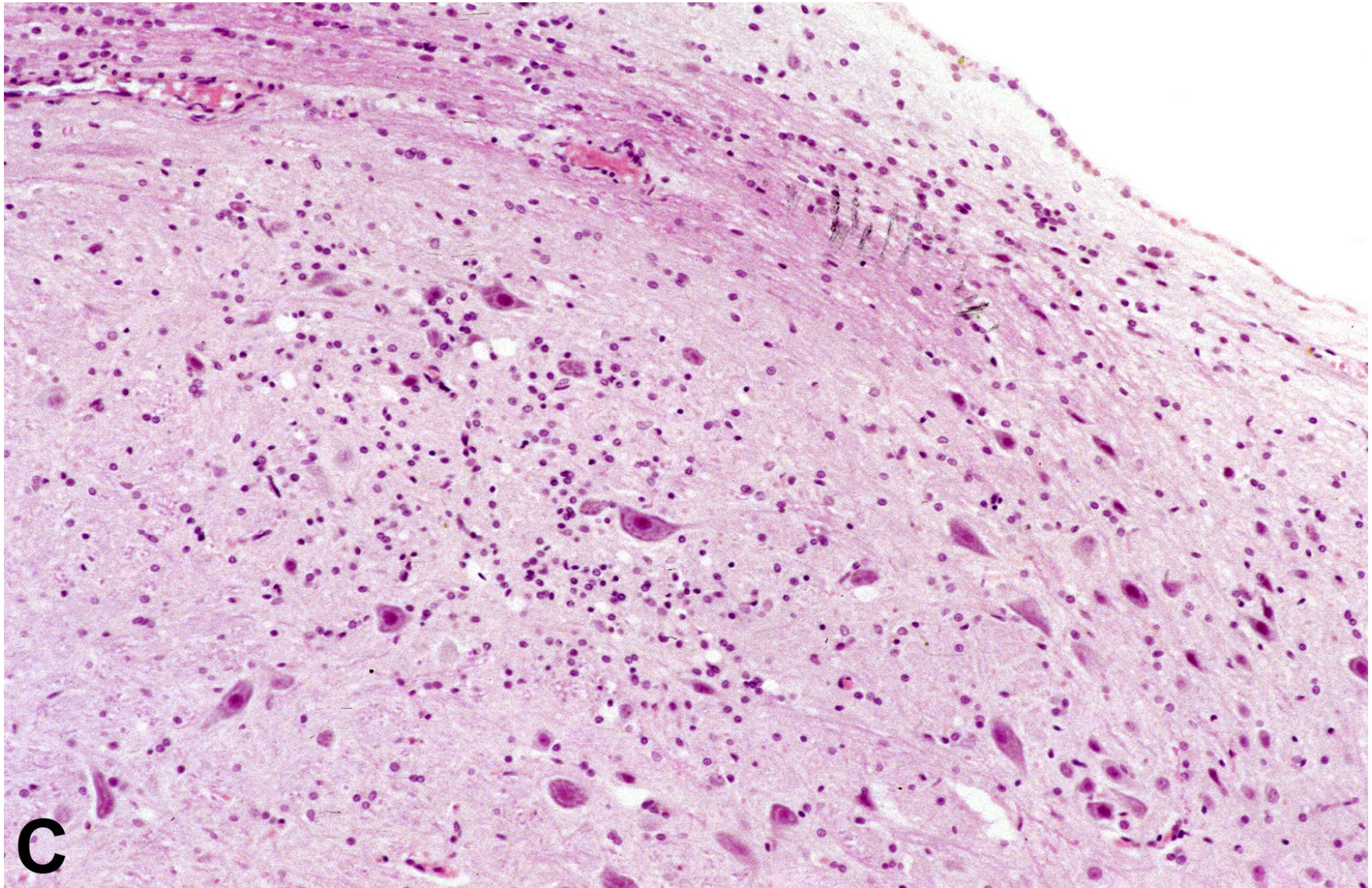


Figure 9C. Medulla. Note vestibular nucleus gliosis. Female rat exposed to 2000 ppm methylmethacrylate by inhalation for 13 weeks. H&E, 40X.

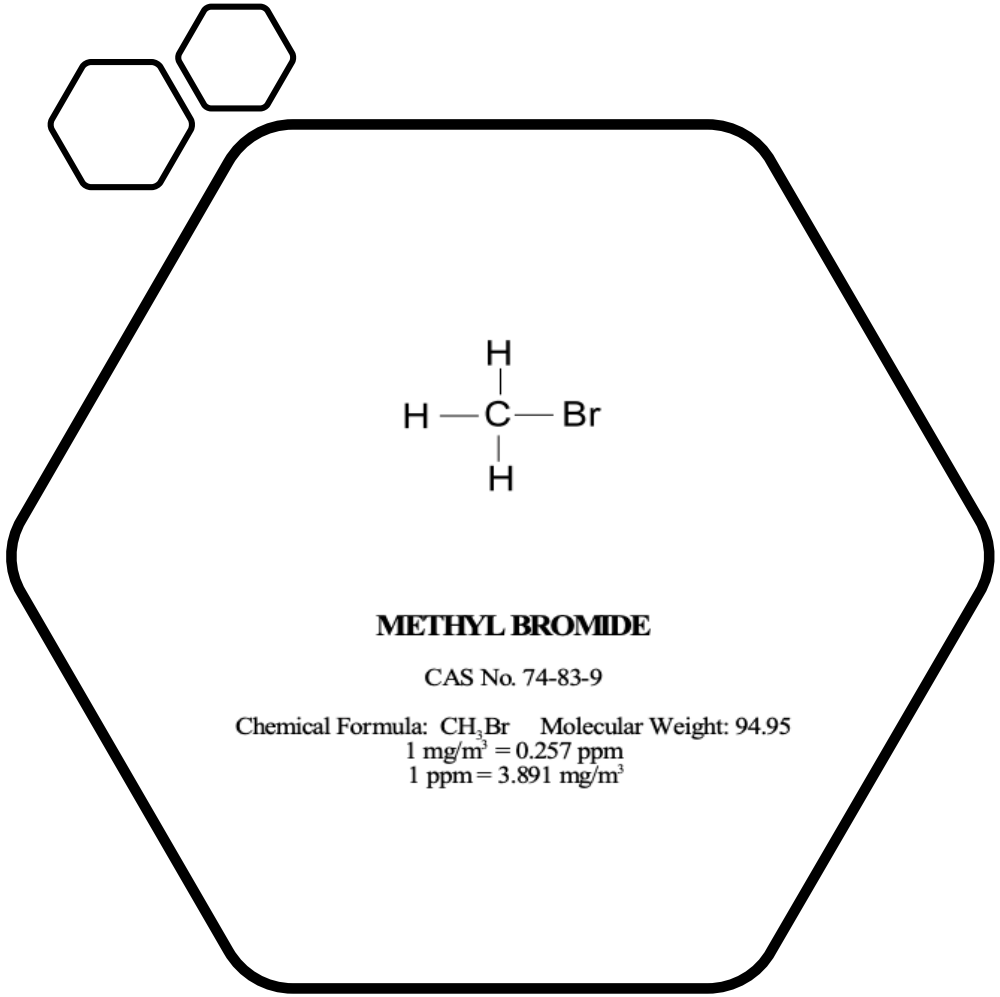


9 Methylmethacrylate Neuropathological diagnoses

- Cerebellum, roof nuclei; neuron, necrosis.
- Vestibular nucleus; hemorrhage.
- Vestibular nucleus; neuron, necrosis.

#10 Methyl Bromide

- An organobromine compound with the formula CH_3Br. It is a colorless, odorless, nonflammable toxic gas produced industrially and biologically. It is a recognized ozone-depleting chemical.
- It is used as a fumigant against insects and rodents in food, tobacco, and nursery stock. Smaller amounts are used in the preparation of other organic compounds.
- Clinical signs: In rats, neurobehavioral testing of high dose males showed some curling and crossing of the hindlimbs whereas females remained unremarkable.
- Now review the following whole slide images: Figure 10-1, Figure 10-2a, Figure 10-2b, and Figure 10-2c.



Methyl Bromide National Toxicology Program, (1992): CNS Lesions

- Gross morphological evaluation of the nervous system was reported to be negative.
- Despite clinical signs, there were no neuropathological lesions evident in this review of Fischer 344 rats as in the original evaluation.
- In mice, neurobehavioral tests indicated high dose males had severe curling and crossing of the hindlimbs and twitching of the forelimbs.
- These signs reached their highest intensity by the 6th week on test. In female dose groups, similar signs were evident but to a lesser degree.
- High dose male mice had minimal to marked neuronal necrosis in the internal granular cell layer of the cerebellum (Figure 10A and Figure 10B).

Methyl Bromide National Toxicology Program, (1992): CNS Lesions

- Neuronal necrosis usually occurred in the dorsal folia of the Ansiform lobe and to a lesser degree in the Flocculonodular lobes when present in tissue sections (see NTP level 6 on slide 12 for cerebellar lobe anatomy).
- In one animal, in which the cerebellum had been sectioned more anteriorly, neuronal necrosis was evident in the culmen (an anterior lobule of the cerebellum). Neuronal necrosis consisted of nuclear pyknosis and karyorrhexis, affecting individual or clusters of granular cells.
- There was no inflammatory reaction to this process.

Figure 10A. Cerebellum. Ansiform lobe from control male mouse. H&E, 100X.

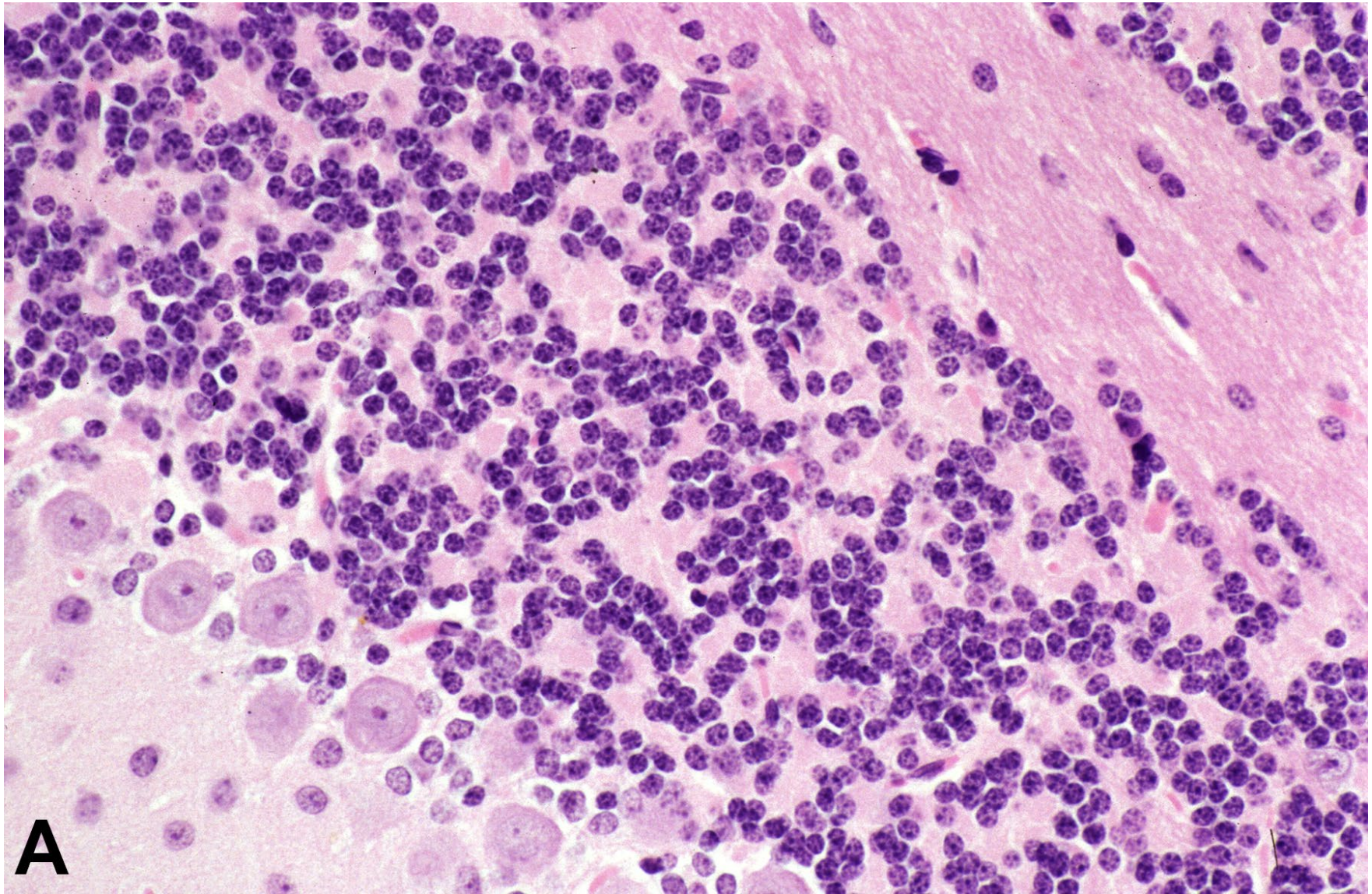
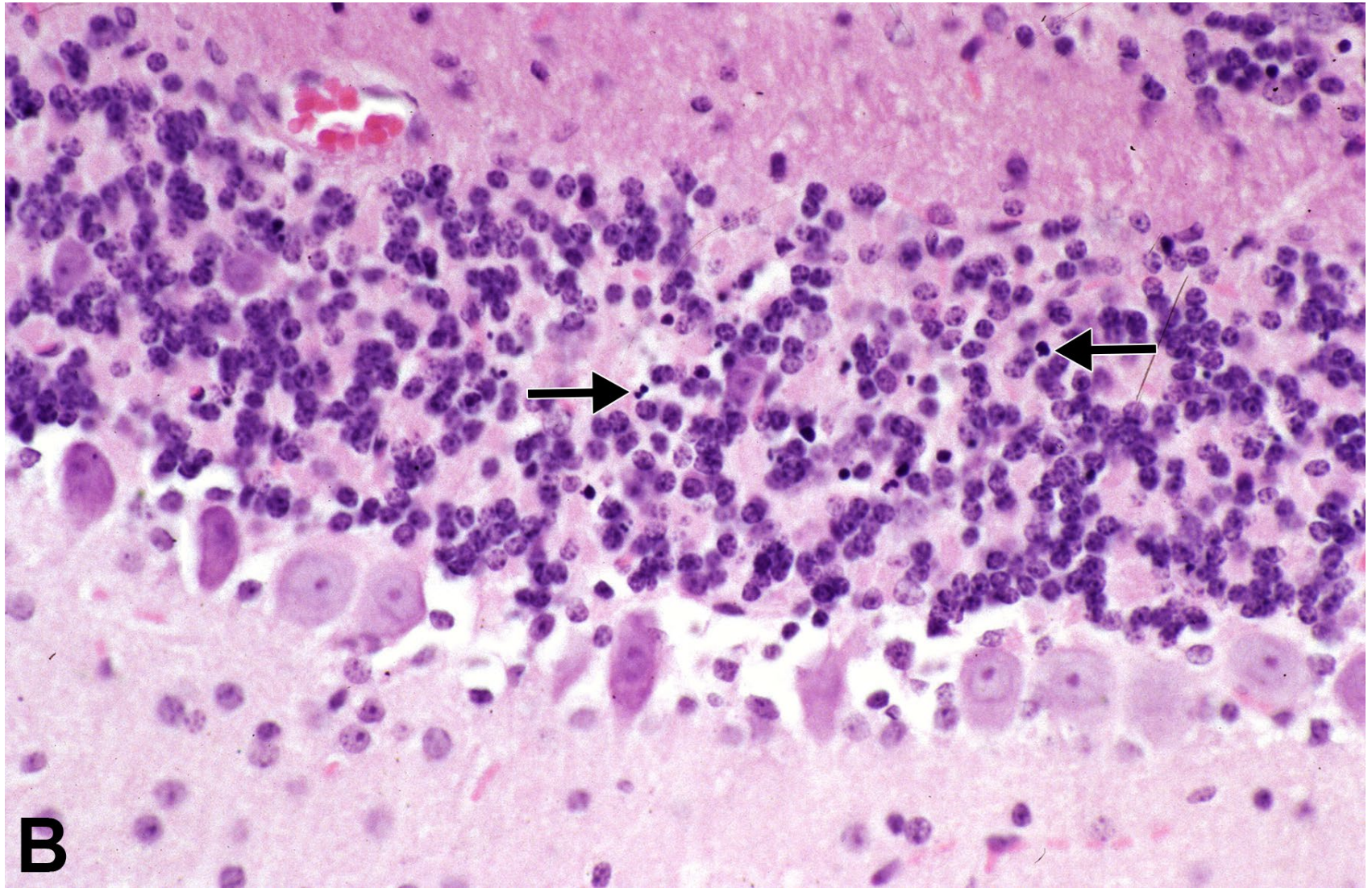


Figure 10B. Cerebellum. Ansiform lobe. Note the scattered pyknotic internal granule cells (arrows). Male mouse exposed to 120 mg/kg methylbromide for 13 weeks. H&E, 100X.



#10 Methyl Bromide Neuropathological diagnosis

- Rats: None evident
- Mice: Cerebellum; internal granule cell layer, neuron, necrosis.

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