Histologic Method for Nonhuman Primate Tooth Evaluation on Toxicity Studies

Sarah Coe[1], Rachel Stoops[2], Chris Bollinger[2], Deanna Buford[2], Matthew Zipprich[2], Susan Kent[3], Judit Markovits[2]

1) Inotiv, Kalamazoo, MI, USA 2) Inotiv, St. Louis, MO, USA3) Inotiv, Evansville, IN, USA

Introduction

Evaluation of nonhuman primate (NHP) teeth is rarely performed in toxicity studies, though may be desired to determine whether test article-related dental lesions observed in rodents are relevant to primates/humans. This study aimed to develop a practical method to process NHP teeth in a high-throughput laboratory for toxicity studies. In order to produce consistent sections, an understanding of normal NHP dentition and tooth eruption times is necessary, especially as NHPs utilized on toxicity studies are frequently juvenile (<5 years of age). The maxillary dentition from a primate approximately three years of age is demonstrated in Figure 1.

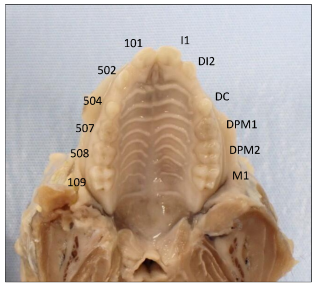


Figure 1. Juvenile Nonhuman Primate Maxillary Dentition

NHPs utilized on toxicity studies are frequently juvenile and are in the process of erupting adult teeth. This primate has both deciduous and permanent teeth. The teeth in this training animal are labeled in the modified Triadan system (left) and with an abbreviated dental formula (right). In the modified Triadan system permanent teeth are labeled with the first numeral "1" in the upper right quadrant, and deciduous teeth are labeled with the first numeral "5" in this quadrant (Gibson et al., 2007). In the dental formula "I" = incisor, "DI"= deciduous incisor, "DPM" = deciduous premolar, and "M" = molar. In a full set of deciduous teeth there are 2 incisors, 1 canine, and 2 premolars per quadrant. In fully mature dentition, there are 2 incisors, 1 canine, 2 premolars, and 3 molars per quadrant (Hillson, 2023).

Methods

- Two colony juvenile Cynomolgus macaques (housed at an AAALAC-accredited CRO in compliance with all federal regulations and Inotiv policies on the care, welfare, and treatment of laboratory animals) were euthanized according to AVMA standards for training purposes, including this method validation. The mandible and maxilla were collected and fixed whole in 10% neutral buffered formalin. An Exakt 312 Diamond Band Pathology Saw was used to produce sections, and technicians wore appropriate PPE to prevent exposure when processing large NHP tissue samples.
- Two sectioning methods were evaluated. Method 1 generated sections sagittal to incisors 101 and 401 and the most caudal upper and lower molar, 109 and 409, respectively (Figure 2). Method 2 adapted standard nasal cavity sections (Chamanza et al., 2016) to generate maxillary molar sections (Figure 3), and the incisor and mandibular sections were generated as in Method 1.
- The samples were decalcified in 7% hydrochloric acid for up to 8 days with two solution changes. The samples were processed and stained routinely with hematoxylin and eosin.
- The sections were reviewed by a pathologist and the criteria considered for quality consisted of: the number of teeth present in 1 plane; the proportion of tooth structures (e.g. unmineralized enamel, ameloblasts, dentin, odontoblasts, pulp cavity, tooth root) visible in 1 plane; presence of artifact (e.g. chatter, hypereosinophilia, vacuolation); and the ease and speed of the method for the histology technicians.

Results

Method 1 produced sections of incisor and molar where dentin, pulp cavity, and tooth root were present in a single plane (Figure 2). Method 1 also captured developing molar tooth buds, which may prove useful in evaluation of test article-related effects on ameloblasts or odontoblasts. Method 2 resulted in sections that had canine and premolar in addition to incisors and molars; however, inconsistent sections of cheek teeth were produced where major structures were not present in a single plane (Figure 3). All histologic sections in Methods 1 and 2 had similar levels of artifact (chatter, folding, etc.). As Method 1 produced fewer blocks and the landmarks were straight forward, technician time spent generating the sections was lower (decreased surface decal, trimming, and QC time). While the sections were more difficult to evaluate due to inconsistent/oblique sections, one strength of Method 2 was that it adapted a technique (nasal turbinate trim) that the lab is already skilled in, which could lead to less training time.

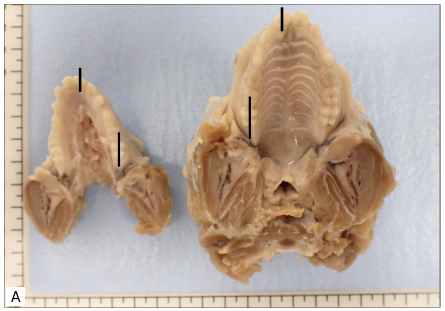
Conclusions

- If microscopic evaluation of NHP teeth is required for a toxicity study, unilateral sagittal sections of an upper and lower incisor and of the most caudal molar present (Method 1) provide practical, high-quality sections for evaluation.
- This method provides enhanced evaluation of oral pathology in NHPs and should be utilized in studies where dental toxicity is suspected based on toxicity evident in other species or mechanism of action of the test article.

References

- Chamanza R, Taylor I, Gregori M, Hill C, Swan M, Goodchild J, Goodchild K, Schofield J, Aldous M, Movat V. Normal Anatomy, Histology, and Spontaneous Pathology of the Nasal Cavity of the Cynomolgus Monkey (Macaca fascicularis). Toxicol Pathol. 2016 Jul;44(5):636-54. doi: 10.1177/0192623315626523. Epub 2016 Mar 2. PMID: 26940715.
- Gibson BW, McGuffey L, Raffo CP, Niemiec BA. Establishing a standardized dental record-keeping system for a small investigational colony of rhesus monkeys (Macaca mulatta). J Med Primatol. 2008 Feb;37 Suppl 1:5-10. doi: 10.1111/j.1600-0684.2007.00257.x. PMID: 18269521.
- Hillson, S. (2023). Dental Anthropology (2nd ed.). Cambridge: Cambridge University Press.

Figure 2. Method 1 of Trimming Nonhuman Primate Teeth for Microscopic Evaluation



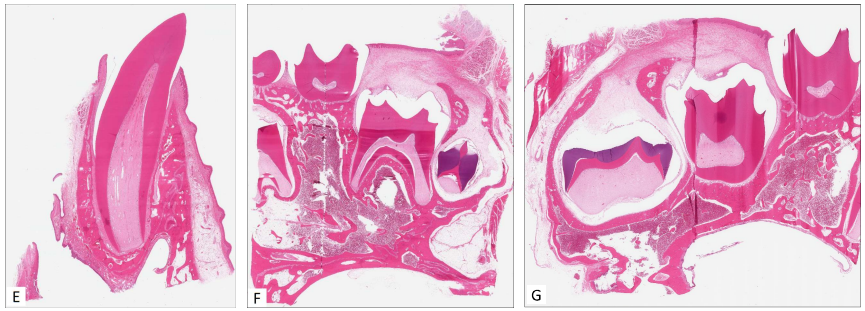
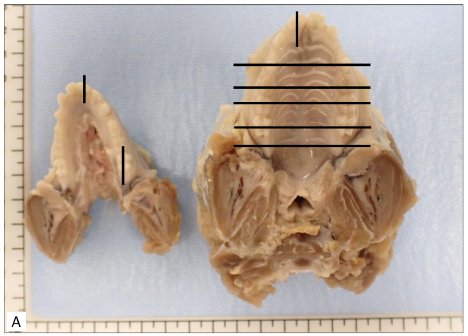
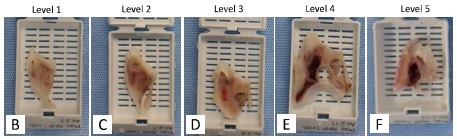


Figure 2. A) The mandible (left) and maxilla (right) of a juvenile nonhuman primate. The lines designate the planes desired for histopathologic evaluation. B) Final trimmed sagittal section of incisor (101) within a standard cassette. C) Final trimmed sagittal section of maxillary molar (109). D) Final trimmed sagittal section of mandibular molar (409). Erupting molars are visible macroscopically in both the maxillary and mandibular trimmed sections. E) Resulting histologic section of incisor. 0.3X. H&E. F) Resulting histologic section of maxillary molar. 0.3X. H&E. G) Resulting histologic section of mandibular molar. 0.3X. H&E. Microscopic structures and cell types visible include full pulp cavity and root of both incisor and molars; ameloblasts, odontoblasts, nonmineralized enamel, dentin, alveolar bone, and gingiva among others. The molar sections included at least one erupted molar and multiple developing teeth. All histologic sections have some level of artifact present, including chatter marks, folding, hypereosinophilia; however, these artifacts do not obscure the main structures. As a total of 4 blocks (only one incisor cassette/block shown here) are generated in this method, the technique was relatively straight forward and limited technician time spent trimming, performing surface decal, performing QC, etc.

Figure 2. Method 2 of Trimming Nonhuman Primate Teeth for Microscopic Evaluation





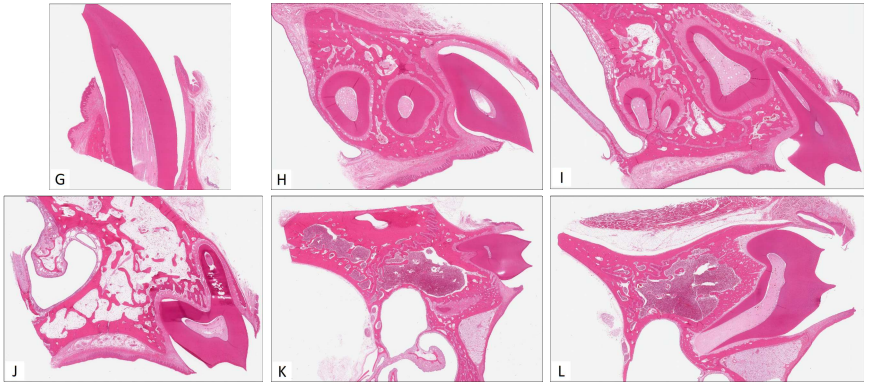


Figure 3. A) The mandible and maxilla of a juvenile NHP. The lines designate the planes desired for histopathologic evaluation. B-F) Final trimmed nasal cross section levels 1-5, respectively. The tissue has been bisected and tissue away from the teeth (i.e. turbinates) have been trimmed away to fit a standard cassette. G) Resulting histologic section of incisor. 0.3X. H&E. Due to the trimming of the nasal cross sections, the root of the incisor has been trimmed away. H-L) Resulting histologic section of maxillary molar, level 1-5, respectively. 0.3X. H&E. Microscopic structures and cell types visible include partial pulp cavity of incisor, canines, premolars, and molars and root of only the premolars or molars; ameloblasts, odontoblasts, nonmineralized enamel, dentin, alveolar bone, and gingiva among others. A total of 8 blocks (only maxillary sections shown here) are generated in this method, making this technique slightly more time consuming.