



January 16, 2025

Donna Hill
US EPA
109 TW Alexander Drive
MD-B105
Research Triangle Park, NC 27711

Reference: Histology and Pathology of Rodent Tissues Following Anatoxin-a Five Day Exposure (Adult CD-1 Mice; June and July 2024 collection), Study ID 68HE0M24P0064, EPL Project No. 431-685, Final Letter Report

Donna Hill:

Background:

Rodent testes were collected from a study designed to evaluate the effects of exposure to a natural cyanotoxin, anatoxin-a. We evaluated a dose response with anatoxin-a given by oral gavage daily for 5 days. A total of 40 male and 40 female adult mice were dosed with either 0, 2, 4, or 6 mg/kg/day in HPLC-grade water as the dosing vehicle.

Testes were collected from 90-95 day old male mice at necropsy 12 days after the last dose. One testis from each mouse was placed in Bouin's for 24 hours and then placed in 70% ethyl alcohol with lithium carbonate for 3 changes until the yellow had dissipated from the tissues and then placed in 70% ethyl alcohol for storage until the histopathology.

The tissue samples were submitted by US EPA to Experimental Pathology Laboratories (EPL®), Inc., Durham, NC. The tissues for all animals were processed routinely for paraffin embedding, sectioned by microtome, mounted on glass slides, stained with hematoxylin and eosin, and covered with glass cover slips. The pathologist examined all slides by brightfield microscopy. Findings were graded from one to five (1=minimal; 2=mild; 3=moderate; 4=marked; and 5=severe), depending upon severity. This report describes the histopathologic findings for the slides submitted for the above-referenced study.

Results:

There were no microscopic findings related to exposure to anatoxin-a. One animal (115) exposed to 6 mg/kg/day had mild vacuolation within the seminiferous tubules.

However, this finding was not observed in any other animal in the study, and so it is unlikely related to exposure to anatoxin-a.

The incidence and severity of all findings are summarized in Appendix A.

Sincerely,

Signed by:
Thomas J. Steinbach
 Signer Name: Thomas J. Steinbach
Signing Reason: I approve this document
Signing Time: 16-Jan-2025 | 2:52:41 PM EST
886140A2A74B420D8D78C907F058D58
Thomas J. Steinbach, DVM
Diplomate, ACVP, ABT
Senior Pathologist

TJS/rg
attachment



QUALITY ASSURANCE FINAL CERTIFICATION

Study Title: Histology and Pathology of Rodent Tissues Following Anatoxin-a Five Day Exposure (Adult CD-1 Mice; June and July 2024 collection)
Client Study: 68HE0M24P0064 EPL Principal Investigator: Dr. Thomas Steinbach

EPL Project Number: 431-685 EPL Pathologist: Dr. Thomas Steinbach

The following aspects of this study were inspected by the Quality Assurance Unit of Experimental Pathology Laboratories, Inc. Dates inspections were performed and findings reported to the EPL Principal Investigator and Management are indicated below.

Area Inspected	Dates	
	Inspection	Reporting
EPL Project Sheet	November 11, 2024	November 11, 2024
Project Setup	November 18, 2024	November 18, 2024
Draft Pathology Report	January 15, 2025	January 15, 2025
Final Pathology Report	January 16, 2025	January 16, 2025

Date reported to Study Director/Management: January 16, 2025

Date of last facility inspection: 11/2024

Signed by:

 Signer Name: Lorri Ezedin
Signing Reason: I am the author of this document
Signing Time: 16-Jan-2025 | 2:57:29 PM EST
 29A1CF2B67DC4E1A89643B9E1E7CA603

EPL Quality Assurance Unit

Date

