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July 22, 1992

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SEQUENCE A

RECEIVED
8/31/92
TCB/ECA

Mr. Terry O'Bryan
Environmental Scientist
U.S. Environmental Protection Agency
TS-778
401 M Street, SW
Washington, DC 20460

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Dear Mr. O'Bryan:

At some point during the past ten years, your organization requested a copy of the final report for CIIT's 90-day vapor inhalation toxicity study of carbon disulfide in Fischer-344 rats (Toxigenics Study No. 420-0711A), Sprague-Dawley rats (Toxigenics Study No. 420-0711B), and B6C3F1 mice (Toxigenics Study No. 420-0711C). The report was released in 1983.

Additional neuropathological examination of the low-dose Fischer-344 and Sprague-Dawley rats from this study was recently completed by Dr. Doyle G. Graham of Duke University. Enclosed are final report amendments for these two studies (420-0711A and 420-0711B).

Cordially,

Willanna A. Griffin

Willanna A. Griffin
Manager, Information Services

Encl.

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FINAL REPORT AMENDMENT NO. 2

FINAL REPORT TITLE: 90-Day Vapor Inhalation Toxicity Study of Carbon Disulfide in Sprague-Dawley Rats (Issued 02/25/83 and amended 04/26/83) (ToxiGenics, Inc. Study No. 420-0711B)

FINAL REPORT AMENDMENT TITLE: Addendum to 90-Day Vapor Inhalation Toxicity Study of Carbon Disulfide in Sprague-Dawley Rats; Subject: Special Neuropathological Examination (ToxiGenics, Inc. Study No. 420-0711B)

FINAL REPORT AMENDMENT DATE: July 10, 1992

Text/Attachment to be Amended: See Addendum Report

Amended to read: See Addendum Report

Reason for Amendment: Additional neuropathological examination of the low dose (T-I) was performed by Dr. Doyle G. Graham (Duke University, Durham, NC) on Sprague-Dawley rats.

James A. Papp
Principal Investigator/Study Director

July 10, 1992
Date

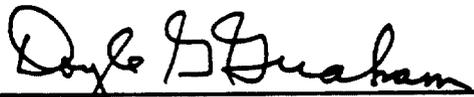
(NOTE: This Amendment is to be maintained with the final report.)

Addendum to
90-DAY VAPOR INHALATION TOXICITY
STUDY OF
CARBON DISULFIDE
IN SPRAGUE-DAWLEY RATS
(ToxiGenics, Inc. Study No. 420-0711B)

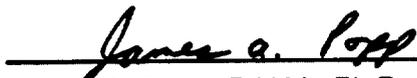
Subject:

SPECIAL NEUROPATHOLOGICAL EXAMINATION

JULY 10, 1992



Doyle G. Graham, M.D., Ph.D.



James A. Popp, D.V.M., Ph.D.
Diplomate, American College of
Veterinary Pathologists
Principal Investigator/Study Director

Introduction

A 90-day inhalation study was conducted in Sprague-Dawley rats, Fischer 344 rats and B6C3F1 mice to determine the subchronic toxicity of carbon disulfide (CS₂) vapors using an exposure regimen of 6 hours per day, 5 days per week for at least 89 consecutive days.

This addendum report contains data pertaining to Sprague-Dawley rats.

The study was performed at ToxiGenics, Inc., 1800 East Pershing Road, Decatur, IL 62526 under the sponsorship of the Chemical Industry Institute of Toxicology, Research Triangle Park, NC 27709. The study began on February 3, 1982 and was completed on May 12, 1982. CIIT issued the original report as CIIT Docket #62063 on February 25, 1983, and it was amended April 26, 1983. The final report was received at CIIT on July 20, 1983. All teased nerve fiber slides were received at CIIT on June 24, 1983. Slides of Epon-embedded tissue were shipped directly to Dr. D. Graham on August 8, 1983 and returned to CIIT on March 25, 1992.

Materials and Methods

Four groups, consisting of 15 male and 15 female Sprague-Dawley rats each and designated as groups U-C, T-I, T-II and T-III, were exposed to atmospheres of carbon disulfide (Pittsburgh Plate and Glass Industries, New Martinsville, WV, 99.9% pure) at target analytical concentrations of 0, 50, 300 and 800 ppm, respectively. The actual time-weighted average concentrations for the treated groups T-I, T-II and T-III were 49.3, 297.1 and 798.4 ppm respectively. No CS₂ was found in the control chamber during the course of the study. Ten rats per group per sex were designated as Principal animals and scheduled for routine clinical and histopathology. The results of this portion of the study have already been presented in CIIT Docket #62063. Five rats per group per sex were designated as Dedicated animals and scheduled for special neuropathology examination.

Dedicated animals were perfused via the left ventricle with 4% phosphate-buffered glutaraldehyde solution following anesthetizing with sodium pentobarbital solution containing approximately 200 units of heparin. The intact perfused animal was refrigerated at approximately 4°C overnight then the right and left sciatic nerve and their branches dissected together with specimens of the cervical and lumbar spinal cord and then placed in 4% glutaraldehyde. The left sural nerve and the large muscle branch of the left tibial nerve were osmicated then placed in cedarwood oil for approximately two weeks. Nerve fibers from the cedarwood oil treated specimens were teased to separate the individual fibers then mounted on glass slides. The teased nerve fibers were coverslipped and retained as permanent specimens. A minimum of 50 teased fibers per rat, approximately 25 per nerve, were prepared. Glutaraldehyde fixed specimens of the right sural nerve, the muscular branch of

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the right tibial nerve, and specimens of the spinal cord from the cervical and lumbar regions were osmicated, dehydrated, and embedded with Epon. Thick sections, longitudinal and cross, of the nerves and cross sections of the spinal cord, were prepared from the Epon specimens and stained with toluidine blue. Other tissues were stored in 10% neutral buffered formalin. Specimens were examined by routine light microscopy for evidence of pathologic change. As specified in the protocol, only UC, T-II, and T-III were examined initially. Group T-I was later examined by Dr. Doyle G. Graham (Duke University Medical Center, Durham, NC) and this new information is contained in this addendum report.

Results

Results of the special neuropathologic studies performed on teased nerve fibers and Epon embedded specimens are summarized in Table 1. Observations made on each rat from groups U-C, T-II and T-III are presented in Appendix A. These results were also presented in the initial study report [CIIT Docket # 62063] as Appendix K and no changes in these results have been made. The observations made on each rat from group T-I are presented in this report, Appendix B and Table 1, and are the result of examination of this group subsequent to the initial study report. Lesions, axonal swelling, and/or clumping and loss of myelin sheaths (degeneration), were present in all five male and female rats from the T-III group in teased fibers of the muscular nerve and in all five male and four of five female rats in teased fibers from the sural nerve. Three T-II male rats exhibited lesions in single fibers, two from the muscular nerve and one from the sural nerve. No lesions were present in the teased fiber preparations from T-II female rats or T-I male or female rats. A similar frequency of lesions were present in Epon sections of the muscular and sural nerves as axonal swelling was present in all five male and female rats from the T-III group. Lesions were not present in Epon sections of the muscular and sural nerves in either the T-I or T-II male or female rats. Axonal swelling was present in the spinal cord in all female and male rats in the T-III group in Epon sections of both lumbar and cervical specimens. Equivocal swelling of axons was noted in the lumbar cord of one T-II male rat, AC5614. Axonal swelling was not noted in the lumbar cord of T-I male or female rats.

Lesions in the teased nerve fibers consisted primarily of a segmental swelling of axons at paranodal and in internodal regions of the fibers. Paranodal swellings occurred both unilaterally or bilaterally around the nodal junctions. In most fibers, the myelin sheaths remained intact; however, in a small percent of the affected fibers, there was axonal degeneration with inclusion of myelin debris within phagocytes. In Epon sections, the axonal swelling was evident by an increase in the diameter of the fibers that were accompanied by thinning of myelin sheaths. This latter change was most easily visualized in longitudinal sections. Occasional macrophages, some with myelin debris within their cytoplasm, were

present in the interstitium. The lesions, both in the number of fibers affected and in severity of the axonal swelling and degeneration, was much more extensive in specimens of the muscular nerve compared to the sural nerve. Lesions in the spinal cord consisted of axonal swelling which were most severe in sections taken from the lumbar area. Occasionally isolated macrophages were present in the cord sections; however, most swollen fibers had intact myelin sheaths and had normal but enlarged axon cylinders. Swollen axons occurred in all funiculi of the cord sections but were most numerous in the ventral and lateral funiculi particularly in sections of the lumbar cord. Fibers with swollen axons were less numerous in the dorsal funiculi and occurred with approximately equal frequency (subjective appraisal) in the cervical and lumbar sections. Specimens, raw data, and final report are stored in the archives of CIIT, Research Triangle Park, NC with the following two exceptions. ToxiGenics raw data is stored on a magnetic tape using a Dec Tops 20 computer system. It is stored in a format for which CIIT has no documentation. CIIT currently has no software/hardware which can retrieve this data in an interpretable format. ToxiGenics, Inc. merged to American Biogenics Corporation in 1985 and closed the laboratory facilities in 1987. CIIT no longer has access to ToxiGenics laboratory facility data.

Summary/Conclusions

Neuropathology evaluation of teased nerve fiber preparations of muscular and sural nerves from male and female Sprague-Dawley rats exposed to carbon disulfide at concentrations of 0, 50, 300 or 800ppm for 90 days revealed axonal swelling of the muscular nerve in all five male and female rats from the T-III (800 ppm) group, and of the sural nerve in all five male and four of five female rats. Three T-II (300 ppm) male rats exhibited lesions in single fibers, two from the muscular nerve and one from the sural nerve. No lesions were present in the teased fiber preparations from T-I male or female or T-II female rats. Axonal swelling of muscular and sural nerves and lumbar and cervical cord fibers was also noted in Epon sections of all five male and female rats from the T-III (800 ppm) group. Lesions were not present in Epon sections of the muscular and sural nerves in either the T-I or T-II male or female rats. Equivocal swelling of axons was noted in the lumbar cord of one T-II male rat.

From these data it is clear that this study establishes 50 ppm as a no effect level for neuropathology of CS₂ in Sprague-Dawley rats.

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**Addendum to 90-Day Vapor Inhalation Toxicity Study of
Carbon Disulfide in Sprague-Dawley Rats**

Subject: Special Neuropathological Examination

- Appendix A** **Special Neurological Study from Study No. 420-0711B, Groups U-C, T-II and T-III**
- Appendix B** **Detailed Neuropathological Findings for T-I Treatment Groups**
- Appendix C** **Quality Assurance Statement**
- Quality Assurance Inspection and Reporting Dates**

Study No. 420-0711B

Appendix A

SPECIAL NEUROLOGICAL STUDY

TABLE K-1: SPECIAL NEUROPATHOLOGIC STUDIES - MALES
 90-DAY VAPOR INHALATION TOXICITY STUDY IN SPRAGUE-DAWLEY RATS
 TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings	
Untreated Control	AC5559	<u>Teased Nerve Fibers (No.)</u>		
		Muscular (32)	Non-remarkable	
		Sural (32)	Non-remarkable	
		<u>Epon</u>		
		Muscular Nerve	Two questionable degenerative fibers on longitudinal section D-1.	
		Sural Nerve	Non-remarkable	
		Lumbar Cord	No changes that can be distinguished from artifacts.	
		Cervical Cord	No changes that can be distinguished from artifacts.	
		AC5575	<u>Teased Nerve Fibers (No.)</u>	
			Muscular (32)	Non-remarkable
			Sural (30)	Non-remarkable
			<u>Epon</u>	
		Muscular Nerve	Non-remarkable	
		Sural Nerve	Non-remarkable	
		Lumbar Cord	No changes that can be distinguished from artifacts.	
		Cervical Cord	No changes that can be distinguished from artifacts.	
	AC5579	<u>Teased Nerve Fibers (No.)</u>		
		Muscular (32)	Non-remarkable	
		Sural (30)	Non-remarkable	
		<u>Epon</u>		
		Muscular Nerve	Non-remarkable	
		Sural Nerve	Non-remarkable	
		Lumbar Cord	No changes that can distinguished from artifacts.	
		Cervical Cord	Poor perfusion. No changes that can be distinguished from artifacts.	
			Poor perfusion.	

TABLE K-1: SPECIAL NEUROPATHOLOGIC STUDIES - MALES
90-DAY VAPOR INHALATION TOXICITY STUDY IN SPRAGUE-DAWLEY RATS
TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
Untreated Control (cont.)	AC5582	<u>Teased Nerve Fibers (No.)</u>	
		Muscular (32)	Non-remarkable
		Sural (32)	Non-remarkable
		<u>Epon</u>	
		Muscular Nerve	Non-remarkable
		Sural Nerve	Non-remarkable
		Lumbar Cord	No changes that can be distinguished from artifacts.
		Cervical Cord	No changes that can be distinguished from artifacts.
	AC5587	<u>Teased Nerve Fibers (No.)</u>	
		Muscular (30)	Non-remarkable
		Sural (31)	Non-remarkable
		<u>Epon</u>	
		Muscular Nerve	A macrophage containing myelin debris is present in a perivascular area (longitudinal section - D-1)
		Sural Nerve	Non-remarkable
		Lumbar Cord	Non-remarkable
		Cervical Cord	Non-remarkable

TABLE K-1: SPECIAL NEUROPATHOLOGIC STUDIES - MALES
 90-DAY VAPOR INHALATION TOXICITY STUDY IN SPRAGUE-DAWLEY RATS
 TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-II	AC5566	<u>Teased Nerve Fibers (No.)</u> Muscular (37)	One fiber shows unequivocal axonal swelling at nodal and internodal areas - mild.
		Sural (36)	One fiber shows a unilateral nodal swelling of minimal severity.
		<u>Epon</u> Muscular Nerve	No definitive lesions
		Sural Nerve	Non-remarkable
		Lumbar Cord	Non-remarkable
		Cervical Cord	Non-remarkable
	AC5569	<u>Teased Nerve Fibers (No.)</u> Muscular (35)	Non-remarkable
		Sural (34)	One fiber shows unequivocal swelling unilaterally at nodal junction.
		<u>Epon</u> Muscular Nerve	Non-remarkable
		Sural Nerve	Non-remarkable
		Lumbar Cord	No changes that can be distinguished from artifacts.
		Cervical Cord	No changes that can be distinguished from artifacts.

TABLE K-1: SPECIAL NEUROPATHOLOGIC STUDIES - MALES
 90-DAY VAPOR INHALATION TOXICITY STUDY IN SPRAGUE-DAWLEY RATS
 TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings	
T-II (cont.)	AC5596	<u>Teased Nerve Fibers (No.)</u>		
		Muscular (37)	Non-remarkable	
		Sural (31)	Non-remarkable	
		<u>Epon</u>		
		Muscular Nerve	Non-remarkable	
		Sural Nerve	Non-remarkable	
		Lumbar Cord	No changes that can be distinguished from artifacts.	
		Cervical Cord	No changes that can be distinguished from artifacts.	
		AC5606	<u>Teased Nerve Fibers (No.)</u>	
		Muscular (32)	One fiber shows severe degeneration, clumping and loss of myelin through several nodes.	
		Sural (32)	Non-remarkable	
		<u>Epon</u>		
	Muscular Nerve	No definitive lesions		
	Sural Nerve	No definitive lesions		
	Lumbar Cord	No definitive lesions		
	Cervical Cord	No definitive lesions		
	AC5614	<u>Teased Nerve Fibers (No.)</u>		
	Muscular (31)	No definitive lesions		
	Sural (32)	Non-remarkable		
	<u>Epon</u>			
	Muscular Nerve	Non-remarkable		
	Sural Nerve	Non-remarkable		
	Lumbar Cord	Equivocal axonal swelling of fibers is present in the lateral funiculi.		
	Cervical Cord	No definitive lesions.		

TABLE K-1: SPECIAL NEUROPATHOLOGIC STUDIES - MALES
90-DAY VAPOR INHALATION TOXICITY STUDY IN SPRAGUE-DAWLEY RATS
TEST ARTICLE: CARBON DISULFIDE

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Group	Animal Number	Specimen	Findings
T-III	AC5551	<u>Teased Nerve Fibers (No.)</u> Muscular (33)	Thirty fibers show nodal and internodal segmental axonal swelling. Myelin clumping is present in occasional fibers.
		Sural (30)	Ten fibers show nodal and/or internodal segmental axonal swelling of moderate degree.
		<u>Epon</u> Muscular Nerve	Numerous fibers show axonal swelling in both longitudinal and cross sections. Macrophages with myelin debris are present in the interstitium.
		Sural Nerve	Small numbers of fibers show axonal swelling in both longitudinal and cross sections.
		Lumbar Cord	Large numbers of fibers in the ventral and lateral funiculi show axonal swelling. Some axons are degenerative and isolated macrophages are visible. Small numbers of fibers with axonal swelling are visible in the dorsal funiculi.

TABLE K-1: SPECIAL NEUROPATHOLOGIC STUDIES - MALES
90-DAY VAPOR INHALATION TOXICITY STUDY IN SPRAGUE-DAWLEY RATS
TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-III (cont.)	AC5551	<u>Epon (cont.)</u> Cervical Cord	Small fibers show axonal swelling in all funiculi.
	AC5567	<u>Teased Nerve Fibers (No.)</u> Muscular (33)	All fibers show nodal and/or internodal axonal swelling. Small numbers of fibers show clumping and loss of myelin.
		Sural (30)	Twenty-three fibers show mild nodal or internodal axonal swelling.
		Muscular Nerve	Large numbers of fibers show swelling on cross sections, smaller numbers on longitudinal sections. Isolated macrophages are present in the interstitium.
		Sural Nerve	Small numbers of fibers show axonal swelling in both cross and longitudinal sections.
	Lumbar Cord	Moderate numbers of fibers in the ventral and lateral funiculi show axonal swelling. Small numbers of fibers are present with swollen axons in the dorsal funiculi.	
	Cervical Cord	Small number of fibers with swollen axons are present in all funiculi.	

TABLE K-1: SPECIAL NEUROPATHOLOGIC STUDIES - MALES
 90-DAY VAPOR INHALATION TOXICITY STUDY IN SPRAGUE-DAWLEY RATS
 TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-III (cont.)	AC5585	<u>Teased Nerve Fibers (No.)</u> Muscular (36)	Thirty-one fibers show segmental nodal and internodal axonal swellings.
		Sural (31)	Nineteen fibers show mild segmental nodal and/or internodal axonal swellings.
		<u>Epon</u> Muscular Nerve	Cross and longitudinal sections show numerous fibers with swollen axons. Macrophages containing myelin debris and degenerative fibers are also present.
		Sural Nerve	Small numbers of fibers in cross and longitudinal sections show mild axonal swellings.
		Lumbar Cord	Ventral and lateral funiculi show moderate numbers of fibers with swollen axons, some of which show vacuolative change. The dorsal funiculi contain small numbers of fibers with swollen axons.
		Cervical Cord	All funiculi show small numbers of fibers with swollen axons.

TABLE K-1: SPECIAL NEUROPATHOLOGIC STUDIES - MALES
90-DAY VAPOR INHALATION TOXICITY STUDY IN SPRAGUE-DAWLEY RATS
TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-III (cont.)	AC5597	<u>Teased Nerve Fibers (No.)</u> Muscular (30)	Sixteen fibers show segmental nodal and/or internodal axonal swelling.
		Sural (32)	Six fibers show mild segmental axonal swelling at internodal or nodal areas.
		<u>Epon</u> Muscular Nerve	Numerous fibers show marked axonal swelling on both cross and longitudinal sections. There are marked variations in branches of the nerve - some branches are edematous and contain macrophages.
		Sural Nerve	Small numbers of fibers in cross and in longitudinal sections show axonal swelling.
		Lumbar Cord	Numerous fibers in the ventral and lateral funiculi show axonal swelling. Small numbers of fibers in the dorsal funiculi show axonal swelling.
		Cervical Cord	Small numbers of fibers in all funiculi show axonal swelling.

TABLE K-1: SPECIAL NEUROPATHOLOGIC STUDIES - MALES
 90-DAY VAPOR INHALATION TOXICITY STUDY IN SPRAGUE-DAWLEY RATS
 TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-III (cont.)	AC5603	<u>Teased Nerve Fibers (No.)</u> Muscular (35)	Twenty-three fibers show nodal and/or paranodal axonal swelling. Occasionally clumping and loss of myelin is visible.
		Sural (38)	Eight fibers show mild nodal or internodal axonal swellings.
		<u>Epon</u> Muscular Nerve	Cross sections show numerous fibers with swollen axons. Longitudinal sections do not show change.
		Sural Nerve	A small number of fibers on both cross and longitudinal sections show axonal swelling.
		Lumbar Cord	Numerous fibers in the ventral and lateral funiculi show axonal swelling. Small numbers of fibers in the dorsal funiculi show axonal swellings.
		Cervical Cord	Small numbers of fibers in the dorsal and lateral funiculi show axonal swelling.

TABLE K-2: SPECIAL NEUROPATHOLOGIC STUDIES - FEMALES
 90-DAY VAPOR INHALATION TOXICITY STUDY IN SPRAGUE-DAWLEY RATS
 TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
Untreated Control	AC5625	<u>Teased Nerve Fibers (No.)</u>	
		Muscular (32)	Non-remarkable
		Sural (31)	Non-remarkable
		<u>Epon</u>	
		Muscular Nerve	Non-remarkable
		Sural Nerve	Non-remarkable
		Lumbar Cord	Non-remarkable
		Cervical Cord	Non-remarkable
	AC5651	<u>Teased Nerve Fibers (No.)</u>	
		Muscular (34)	Non-remarkable
		Sural (32)	Non-remarkable
		<u>Epon</u>	
		Muscular Nerve	Longitudinal section - D1 shows fibers with unilateral axonal swelling at nodal junction.
		Sural Nerve	Non-remarkable
		Lumbar Cord	Non-remarkable
		Cervical Cord	No changes that can be distinguished from artifacts.
	AC5659	<u>Teased Nerve Fibers (No.)</u>	
		Muscular (33)	Non-remarkable
		Sural (33)	Non-remarkable
		<u>Epon</u>	
		Muscular Nerve	Non-remarkable
		Sural Nerve	Non-remarkable
		Lumbar Cord	No changes that can be distinguished from artifacts.
		Cervical Cord	No changes that can be distinguished from artifacts.

TABLE K-2: SPECIAL NEUROPATHOLOGIC STUDIES - FEMALES
 90-DAY VAPOR INHALATION TOXICITY STUDY IN SPRAGUE-DAWLEY RATS
 TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
Untreated Control (cont.)	AC5685	<u>Teased Nerve Fibers (No.)</u>	
		Muscular (33)	Non-remarkable
		Sural (32)	Non-remarkable
		<u>Epon</u>	
		Muscular Nerve	Non-remarkable
		Sural Nerve	One fiber shows a swollen axon, section - A2.
		Lumbar Cord	Some variation in the size of the fibers.
		Cervical Cord	Non-remarkable
	AC5692	<u>Teased Nerve Fibers (No.)</u>	
		Muscular (42)	Non-remarkable
		Sural (32)	Non-remarkable
		<u>Epon</u>	
		Muscular Nerve	Non-remarkable
		Sural Nerve	Non-remarkable
		Lumbar Cord	No changes that can be distinguished from artifacts.
		Cervical Cord	No changes that can be distinguished from artifacts.

TABLE K-2: SPECIAL NEUROPATHOLOGIC STUDIES - FEMALES
90-DAY VAPOR INHALATION TOXICITY STUDY IN SPRAGUE-DAWLEY RATS

TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-II	AC5633	<u>Teased Nerve Fibers (No.)</u>	
		Muscular (31)	Non-remarkable
		Sural (36)	Non-remarkable
		<u>Epon</u>	
		Muscular Nerve	Non-remarkable
		Sural Nerve	Non-remarkable
		Lumbar Cord	No definitive lesions
		Cervical Cord	No definitive lesions
	AC5652	<u>Teased Nerve Fibers (No.)</u>	
		Muscular (32)	Non-remarkable
		Sural (35)	No definitive lesions
		<u>Epon</u>	
	Muscular Nerve	No definitive lesions	
	Sural Nerve	No definitive lesions	
	Lumbar Cord	No definitive lesions	
	Cervical Cord	No definitive lesions	
AC5656	<u>Teased Nerve Fibers (No.)</u>		
	Muscular (32)	Non-remarkable	
	Sural (32)	Non-remarkable	
	<u>Epon</u>		
	Muscular Nerve	No definitive lesions	
	Sural Nerve	No definitive lesions	
	Lumbar Cord	No changes that can be distinguished from artifacts.	
	Cervical Cord	No changes that can be distinguished from artifacts.	
AC5665	<u>Teased Nerve Fibers (No.)</u>		
	Muscular (33)	Non-remarkable	
	Sural (32)	Non-remarkable	
	<u>Epon</u>		
	Muscular Nerve	Non-remarkable	
	Sural Nerve	Non-remarkable	
	Lumbar Cord	No definitive lesions	
	Cervical Cord	No definitive lesions	

TABLE K-2: SPECIAL NEUROPATHOLOGIC STUDIES - FEMALES
90-DAY VAPOR INHALATION TOXICITY STUDY IN SPRAGUE-DAWLEY RATS
TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-II (cont.)	AC5678	<u>Teased Nerve Fibers (No.)</u>	
		Muscular (32)	Non-remarkable
		Sural (36)	Non-remarkable
		<u>Epon</u>	
		Muscular Nerve	Non-remarkable
		Sural Nerve	Non-remarkable
		Lumbar Cord	Non-remarkable
		Cervical Cord	Non-remarkable

TABLE K-2: SPECIAL NEUROPATHOLOGIC STUDIES - FEMALES
 90-DAY VAPOR INHALATION TOXICITY STUDY IN SPRAGUE-DAWLEY RATS
 TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-III	AC5635	<u>Teased Nerve Fibers (No.)</u> Muscular (32)	Nine fibers show mild segmental swelling at nodes and/or internodal regions. One fiber shows minimal axonal swelling on internodal area. Two other fibers show questionable swelling.
		Sural (31)	
		<u>Epon</u> Muscular Nerve	Moderate numbers of fibers show axonal swelling in cross sections and small numbers in longitudinal sections - with marked variations in different bundles.
		Sural Nerve	Small numbers of fibers with swollen axons are present in both longitudinal and cross sections - marked variation in the different bundles.
		Lumbar Cord	Moderate numbers of fibers in the ventral and lateral funiculi show axonal swellings. Questionable swelling of axons is visible in the dorsal funiculi.

TABLE K-2: SPECIAL NEUROPATHOLOGIC STUDIES - FEMALES
 90-DAY VAPOR INHALATION TOXICITY STUDY IN SPRAGUE-DAWLEY RATS
 TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-III (cont.)	AC5635	<u>Epon</u> (cont.) Cervical Cord	Small numbers of fibers in all funiculi with swollen axons are present.
	AC5654	<u>Teased Nerve Fibers</u> (No.) Muscular (30)	Twenty-one fibers show segmental axonal swelling at nodal and/or internodal regions. Two additional fibers show clumping and loss of myelin.
		Sural (33)	Six fibers show minimal segmental nodal and/or axonal swelling. An additional fiber shows clumping and loss of myelin.
		<u>Epon</u> Muscular Nerve	Moderate numbers of fibers show axonal swelling in cross and longitudinal sections.
		Sural Nerve	Small numbers of fibers show axonal swelling in cross and longitudinal sections.
		Lumbar Cord	Moderate numbers of fibers in the ventral and lateral funiculi show swollen axons. Isolated fibers in the dorsal funiculi have swollen axons.

TABLE K-2: SPECIAL NEUROPATHOLOGIC STUDIES - FEMALES
90-DAY VAPOR INHALATION TOXICITY STUDY IN SPRAGUE-DAWLEY RATS
TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-III (cont.)	AC5654	<u>Epon (cont.)</u> Cervical Cord	Small numbers of fibers in all funiculi show swollen axons.
	AC5669	<u>Teased Nerve Fibers (No.)</u> Muscular (40)	Thirty-four fibers show segmental axonal swelling at nodes and/or internodal regions. Three additional fibers show clumping and loss of myelin.
		Sural (32)	Eight fibers show mild segmental nodal and/or internodal axonal swelling. Two fibers show clumping and loss of myelin.
		<u>Epon</u> Muscular Nerve	Numerous fibers in cross and longitudinal sections show axonal swellings and scattered macrophages with myelin debris.
		Sural Nerve	Low to moderate numbers of fibers in cross and longitudinal sections show axonal swelling.

TABLE K-2: SPECIAL NEUROPATHOLOGIC STUDIES - FEMALES
90-DAY VAPOR INHALATION TOXICITY STUDY IN SPRAGUE-DAWLEY RATS
TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-III (cont.)	AC5669	<u>Epon (cont.)</u> Lumbar Cord	Moderate numbers of fibers in the lateral and ventral funiculi show swollen axons with some myelin debris. Dorsal funiculi show small numbers of swollen axons.
		Cervical Cord	Small numbers of fibers in all funiculi show swollen axons.
		AC5674 <u>Teased Nerve Fibers (No.)</u> Muscular (31)	Twenty-eight fibers show segmental nodal and/or internodal axonal swelling. Occasional clumping and loss of myelin of fibers is visible.
		Sural (30)	Two fibers show minimal axonal swelling.
		<u>Epon</u> Muscular Nerve	Moderate numbers of fibers in cross sections show axonal swelling. Questionable swelling of fibers is present in longitudinal sections.
		Sural Nerve	Small numbers of fibers in cross and longitudinal sections show axonal swelling.

TABLE K-2: SPECIAL NEUROPATHOLOGIC STUDIES - FEMALES
 90-DAY VAPOR INHALATION TOXICITY STUDY IN SPRAGUE-DAWLEY RATS
 TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-III (cont.)	AC5674	<u>Epon (cont.)</u> Lumbar Cord	Small to moderate numbers of fibers are present in the ventral and lateral funiculi. Questionable axonal swelling is visible in the dorsal funiculi.
		Cervical Cord	Small numbers of fibers with swollen axons are present in all funiculi.
	AC5702	<u>Teased Nerve Fibers (No.)</u> Muscular (32)	Twenty-five fibers show segmental axonal swelling at nodes and/or internodal regions. One fiber shows clumping and loss of myelin.
		Sural (35)	Non-remarkable
		<u>Epon</u> Muscular Nerve	Numerous fibers in cross sections show axonal swellings. Small numbers of fibers are visible with axonal swelling in longitudinal sections. Some bundles appear edematous.

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TABLE K-2: SPECIAL NEUROPATHOLOGIC STUDIES - FEMALES
90-DAY VAPOR INHALATION TOXICITY STUDY IN SPRAGUE-DAWLEY RATS
TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-III (cont.)	AC5702	<u>Epon</u> (cont.) Sural Nerve	Small to moderate number of fibers in cross section show axonal swelling - marked variation between bundles. Small numbers of fibers are visible in longitudinal sections with swollen axons.
		Lumbar Cord	Moderate number of fibers with swollen axons are present in the lateral and ventral funiculi. Isolated fibers in the dorsal funiculi have swollen axons.
		Cervical Cord	Small numbers of fibers with swollen axons are present in all funiculi.

Appendix B

Study 420-0711B (Sprague-Dawley Rats)

Detailed Neuropathological Findings

for T-I Treatment Group (50 ppm)

Test Article: Carbon Disulfide

Study 420-0711B (Sprague-Dawley Rats)

90-Day Vapor Inhalation Toxicity Study in Sprague-Dawley Rats

Detailed Neuropathological Findings

T-I Treatment Group (50 ppm)

Test Article: Carbon Disulfide

Animal Number	Sex	Teased Fiber Study		Epon Sections			
		Muscular N.	Sural N.	Muscular N.	Sural N.	Lumbar Cord	Cervical Cord
AC5542	M	0	0	0	0	0	0
AC5543	M	0	0	0	0	0	0
AC5592	M	0	0	0	0	0	0
AC5604	M	0	0	0	0	0	0
AC5605	M	0	0	0	0	0	0
AC5627	F	0	0	0	0	0	0
AC5661	F	0	0	0	0	0	0
AC5666	F	0	0	0	0	0	0
AC5673	F	0	0	0	0	0	0
AC5699	F	0	0	0	0	0	0

Teased nerve fibers and epon sections were examined for evidence of axonal swellings or degeneration. Lesions were absent from this treatment group.

Appendix C

Quality Assurance Statement

Quality Assurance Inspection and Reporting Dates

CHEMICAL INDUSTRY INSTITUTE OF TOXICOLOGY
6 Davis Drive, P.O. Box 12137
Research Triangle Park, NC 27709

QUALITY ASSURANCE STATEMENT

STUDY TITLE: Addendum to 90-Day Vapor Inhalation Toxicity Study of Carbon Disulfide in Sprague-Dawley Rats; Subject: Special Neuropathological Examination (ToxiGenics, Inc. Study No. 420-0711B)

The conduct of this study was subjected at ToxiGenics, Inc. and Microbiological Associates to periodic Quality Assurance inspections. The dates of inspections and the dates that findings were reported to the Study Director and Management were included in CIIT Docket 62063, page 4 (Vol. I) and page 157 (Vol. III), respectively.

This addendum report has been reviewed by Chemical Industry Institute of Toxicology's Quality Assurance in accordance with FDA's Good Laboratory Practice Regulation, CFR 21 Part 58. The dates of inspection and the dates that findings were reported to the Study Director/Management are attached to this report.

Patricia O'Brien Pomerleau
Patricia O'Brien Pomerleau, M.S.
Quality Assurance Manager

07/08/92
Date

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CHEMICAL INDUSTRY INSTITUTE OF TOXICOLOGY
6 Davis Drive, P.O. Box 12137
Research Triangle Park, NC 27709

QUALITY ASSURANCE INSPECTION AND REPORTING DATES

Study Title: Addendum to 90-Day Vapor Inhalation Toxicity Study of Carbon Disulfide in Sprague-Dawley Rats; Subject: Special Neuropathological Examination (ToxiGenics, Inc. Study No. 420-0711B)

Dates of
Inspection

08/19-20/91
09/18/91
01/20/92,
02/24/92,
02/27-28/92,
03/04/92,
&
03/17&20/92
07/08/92

Dates of Report
to Study Director/Management

08/21/91
09/19/91
03/23/92
07/08/92

05/10/1992

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CHEMICAL INDUSTRY INSTITUTE OF TOXICOLOGY
P.O. BOX 12137
6 DAVIS DRIVE
RESEARCH TRIANGLE PARK, NC 27709

FINAL REPORT AMENDMENT NO. 2

FINAL REPORT TITLE: 90-Day Vapor Inhalation Toxicity Study of Carbon Disulfide in Fischer 344 Rats (Issued 02/25/83 and amended 04/26/83) (ToxiGenics, Inc. Study No. 420-0711A)

FINAL REPORT AMENDMENT TITLE: Addendum to 90-Day Vapor Inhalation Toxicity Study of Carbon Disulfide in Fischer 344 Rats; Subject: Special Neuropathological Examination (ToxiGenics, Inc. Study No. 420-0711A)

FINAL REPORT AMENDMENT DATE: July 10, 1992

Text/Attachment to be Amended: See Addendum Report

Amended to read: See Addendum Report

Reason for Amendment: Additional neuropathological examination of the low dose (I-I) was performed by Dr. Doyle G. Graham (Duke University, Durham, NC) on Fischer 344 rats.

James A. Pott
Principal Investigator/Study Director

July 10, 1992
Date

(NOTE: This Amendment is to be maintained with the final report.)

Addendum to
90-DAY VAPOR INHALATION TOXICITY
STUDY OF
CARBON DISULFIDE
IN FISCHER 344 RATS
(ToxiGenics, Inc. Study No. 420-0711A)

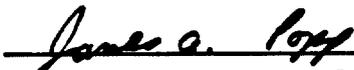
Subject:

SPECIAL NEUROPATHOLOGICAL EXAMINATION

JULY 10, 1992



Doyle G. Graham, M.D., Ph.D.



James A. Popp, D.V.M., Ph.D.

Diplomate, American College of
Veterinary Pathologists

Principal Investigator/Study Director

Introduction

A 90-day inhalation study was conducted in Fischer 344 rats, Sprague-Dawley rats, and B6C3F1 mice to determine the subchronic toxicity of carbon disulfide (CS₂) vapors using an exposure regimen of 6 hours per day, 5 days per week for at least 89 consecutive days.

This addendum report contains data pertaining to Fischer 344 rats.

The study was performed at ToxiGenics, Inc., 1800 East Pershing Road, Decatur, IL 62526 under the sponsorship of the Chemical Industry Institute of Toxicology (CIIT), Research Triangle Park, NC 27709. The study began on February 3, 1982 and was completed on May 12, 1982. CIIT issued the original report as CIIT Docket #52063 on February 25, 1983, and as amended April 26, 1983. The final report was received at CIIT on July 20, 1983. All teased nerve fiber slides were received at CIIT on June 24, 1983. Slides of Epon-embedded tissue were shipped directly to Dr. D. Graham on August 8, 1983 and returned to CIIT on March 25, 1992.

Materials and Methods

Four groups, consisting of 15 male and 15 female Fischer 344 rats each and designated as groups U-C, T-I, T-II and T-III, were exposed to atmospheres of carbon disulfide (Pittsburgh Plate and Glass Industries, New Martinsville, WV, 99.9% pure) at target analytical concentrations of 0, 50, 300 and 800 ppm, respectively. The actual time-weighted average concentrations for the treated groups T-I, T-II and T-III were 49.3, 297.1 and 798.4 ppm, respectively. No CS₂ was found in the control chamber during the course of the study. Ten rats per group per sex were designated as Principal animals and scheduled for routine clinical and histopathology. The results of this portion of the study have already been presented in CIIT Docket #52063. Five rats per group per sex were designated as Dedicated animals and scheduled for special neuropathology examination.

Dedicated animals were perfused via the left ventricle with 4% phosphate-buffered glutaraldehyde solution following anesthetizing with sodium pentobarbital solution containing approximately 200 units of heparin. The intact perfused animal was refrigerated at approximately 4°C overnight then the right and left sciatic nerve and their branches dissected together with specimens of the cervical and lumbar spinal cord and then placed in 4% glutaraldehyde. The left sural nerve and the large muscle branch of the left tibial nerve were osmicated then placed in cedarwood oil for approximately two weeks. Nerve fibers from the cedarwood oil-treated specimens were teased to separate the individual fibers then mounted on glass slides. The teased nerve fibers were coverslipped and retained as permanent specimens. A minimum of 50 teased fibers per rat, approximately 25 per nerve, were prepared. Glutaraldehyde fixed specimens of the right sural nerve, the muscular branch of the right tibial nerve, and specimens of the spinal cord from the cervical and lumbar regions were osmicated, dehydrated, and embedded with Epon. Thick sections, longitudinal and cross, of the nerves and cross sections of the spinal cord, were prepared from the Epon specimens and stained with

toluidine blue. Other tissues were stored in 10% neutral buffered formalin. Specimens were examined by routine light microscopy for evidence of pathologic change. As specified in the protocol, only UC, T-II, and T-III were examined initially. Group T-I was later examined by Dr. Doyle G. Graham (Duke University Medical Center, Durham, NC) and this new information is contained in this addendum report.

Results

Results of the special neuropathologic studies performed on teased nerve fibers and Epon embedded specimens are summarized in Table 1. Observations made on each rat from groups U-C, T-II and T-III are presented in Appendix A. These results were also presented in the initial study report [CIIT Docket # 52063] as Appendix K and no changes in these results have been made. The observations made on each rat from group T-I are presented in this report, Appendix B and Table 1, and are the result of examination of this group subsequent to the initial study report. Lesions, axonal swelling, and/or clumping and loss of myelin sheaths (degeneration), were present in all five male and female rats from the T-III group in teased fibers of the muscular nerve and in all five male and three of five female rats in teased fibers from the sural nerve. One T-II female rat exhibited an equivocal lesion in a single fiber from the sural nerve. No lesions were present in the teased fiber preparations from T-II male rats or T-I male or female rats. A similar frequency of lesions was present in Epon sections. Axonal swelling was present in the muscular nerve of all five male and female rats from the T-III group and in the sural nerve of all five male and four female rats from the T-III group. Axonal swelling was also present in Epon sections of the muscular nerve in two of five females in the T-II group. No lesions were present in the Epon nerve sections from rats in the T-I group. Axonal swelling was present in the spinal cord in all female and male rats in the T-III group in Epon sections of both lumbar and cervical specimens. No definitive lesions were present in the spinal cord sections of T-II, T-I or control rats.

Lesions in the teased nerve fibers consisted primarily of a segmental swelling of axons at paranodal and in internodal regions of the fibers. Paranodal swellings occurred both unilaterally or bilaterally around the nodal junctions. In most fibers, the myelin sheaths remained intact; however, in a small percent of the affected fibers, there was axonal degeneration with inclusion of myelin debris within phagocytes. In Epon sections, the axonal swelling was evident by an increase in the diameter of the fibers that were accompanied by thinning of myelin sheaths. This latter change was most easily visualized in longitudinal sections. Occasional macrophages, some with myelin debris within their cytoplasm, were present in the interstitium. The lesions, both in the number of fibers affected and in severity of the axonal swelling and degeneration, was much more extensive in specimens of the muscular nerve compared to the sural nerve. Lesions in the spinal cord consisted of axonal swelling which were most severe in sections taken from the lumbar area. Occasionally isolated macrophages were present in the cord sections; however, most swollen fibers had intact myelin sheaths and had normal but

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enlarged axon cylinders. Swollen axons occurred in all funiculi of the cord sections but were most numerous in the ventral and lateral funiculi particularly in sections of the lumbar cord. Fibers with swollen axons were less numerous in the dorsal funiculi and occurred with approximately equal frequency (subjective appraisal) in the cervical and lumbar sections. Specimens, raw data, and final report are stored in the archives of CIIT, Research Triangle Park, NC with the following two exceptions. ToxiGenics raw data is stored on a magnetic tape using a Dec Tops 20 computer system. It is stored in a format for which CIIT has no documentation. CIIT currently has no software/hardware which can retrieve this data in an interpretable format. ToxiGenics, Inc. merged to American Biogenics Corporation in 1985 and closed the laboratory facilities in 1987. CIIT no longer has access to ToxiGenics laboratory facility data.

Summary/Conclusions

Neuropathology evaluation of teased nerve fiber preparations of muscular and sural nerves from male and female Fischer 344 rats exposed to carbon disulfide at concentrations of 0, 50, 300 or 800ppm for 90 days revealed axonal swelling of the muscular nerve in all five male and female rats from the T-III (800 ppm) group, and of the sural nerve in all five male and three of five female rats. One T-II (300 ppm) female rat exhibited an equivocal lesion in a single fiber from the sural nerve. No lesions were present in the teased fiber preparations from T-I male or female or T-II male rats. Axonal swelling of muscular and sural nerves and lumbar and cervical cord fibers was also noted in Epon sections of all five male and female rats from the T-III (800 ppm) group, except for the sural nerve in one female rat. Axonal swelling was also observed in Epon sections of the muscular nerve in two of five females in the T-II group. Lesions were not present in the muscular and sural nerves, or the cervical and lumbar spinal cord, in the T-I and control groups.

From these data it is clear that this study establishes 50 ppm as a no effect level for neuropathology of CS₂ in Fischer 344 rats.

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**Addendum to 90-Day Vapor Inhalation Toxicity Study of
Carbon Disulfide in Fischer 344 Rats**

Subject: Special Neuropathological Examination

- | | |
|-------------------|--|
| Appendix A | Special Neurological Study from Study No. 420-0711A, Groups U-C, T-II and T-III |
| Appendix B | Detailed Neuropathological Findings for T-I Treatment Groups |
| Appendix C | Quality Assurance Statement |
| | Quality Assurance Inspection and Reporting Dates |

Study No. 420-0711A

Appendix A

SPECIAL NEUROLOGICAL STUDY

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TABLE K-1: SPECIAL NEUROPATHOLOGIC STUDIES - MALES
90-DAY VAPOR INHALATION TOXICITY STUDY
IN FISCHER 344 RATS

TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings	
Untreated Control	AC5390	<u>Teased Nerve Fibers</u> Muscular (30)	Non-remarkable	
		Sural (33)	Non-remarkable	
	Epon	Muscular Nerve	Sural Nerve	Non-remarkable
			Lumbar Cord	Non-remarkable
		Cervical Cord	Dorsal funiculi show multiple dilated but intact myelin sheaths - probably fixation artifact	Non-remarkable
				Non-remarkable
AC5418	<u>Teased Nerve Fibers</u> Muscular (31)	Sural (34)	Fifteen fibers show minimal, dark paranodal, unilateral enlargements, non-pathologic	
			Non-remarkable	
	Epon	Muscular Nerve	Non-remarkable	
		Sural Nerve	Non-remarkable	
AC5423	<u>Teased Nerve Fibers</u> Muscular (28)	Lumbar Cord	Non-remarkable	
		Cervical Cord	Non-remarkable	
		Sural (30)	Numerous fibers show slight dark enlargements at nodal junctions, bilaterally and laterally, non-pathologic	
			Non-remarkable	

TABLE K-1: SPECIAL NEUROPATHOLOGIC STUDIES - MALES
 90-DAY VAPOR INHALATION TOXICITY STUDY
 IN FISCHER 344 RATS
 TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
Untreated Control (cont.)	AC5423 (cont.)	<u>Epon</u> Muscular Nerve	Non-remarkable
		Sural Nerve	Non-remarkable
		Lumbar Cord	Marked variation of size of axons in dorsal funiculi
		Cervical Cord	Non-remarkable
	AC5439	<u>Teased Nerve Fibers</u> Muscular (29)	Non-remarkable
		Sural (32)	Non-remarkable
	AC5453	<u>Epon</u> Muscular Nerve	Non-remarkable
		Sural Nerve	Non-remarkable
		Lumbar Cord	Non-remarkable
		Cervical Cord	Non-remarkable
		<u>Teased Nerve Fibers</u> Muscular (34)	Four fibers show slight dark unilateral enlargement at nodal junctions, non-pathologic
			Sural (31)
	<u>Epon</u> Muscular Nerve	Non-remarkable	
		Sural Nerve	Non-remarkable
	Lumbar Cord	Some variation in fiber and axon size in dorsal funiculi - questionable if swollen	
	Cervical Cord	Non-remarkable	

TABLE K-1: SPECIAL NEUROPATHOLOGIC STUDIES - MALES

90-DAY VAPOR INHALATION TOXICITY STUDY
IN FISCHER 344 RATS

TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings	
T-II	AC5386	<u>Teased Nerve Fibers (No.)</u>		
		Muscular (34)	No distinct lesions. One fiber shows a suggestion of a unilateral paranodal swelling - interpreted as an artifact.	
		Sural (34)	Non-remarkable	
		<u>Epon</u>		
		Muscular Nerve	No definitive lesions	
		Sural Nerve	No definitive lesions	
		Lumbar Cord	No definitive lesions that can be distinguished from artifacts.	
		Cervical Cord	No definitive lesions that can be distinguished from artifacts.	
		AC5391	<u>Teased Nerve Fibers (No.)</u>	
		Muscular (33)	No definitive lesions. One fiber shows questionable unilateral swelling at a node.	
Sural (32)	Non-remarkable			
	<u>Epon</u>			
	Muscular Nerve	Non-remarkable		
	Sural Nerve	Non-remarkable		
	Lumbar Cord	Non-remarkable		
	Cervical Cord	Non-remarkable		
AC5413	<u>Teased Nerve Fibers (No.)</u>			
	Muscular (31)	Non-remarkable		
	Sural (33)	Three fibers show highly questionable unilateral nodal swelling. Not as definitive lesions.		

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TABLE K-1: SPECIAL NEUROPATHOLOGIC STUDIES - MALES
 90-DAY VAPOR INHALATION TOXICITY STUDY
 IN FISCHER 344 RATS

TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-II (cont.)	AC5413 (cont.)	<u>Epon</u> Muscular Nerve	Non-remarkable
		Sural Nerve Lumbar Cord	Non-remarkable No definitive changes from animals.
		Cervical Cord	No definitive changes from control animals.
	AC5435	<u>Teased Nerve Fibers (No.)</u> Muscular (29)	Non-remarkable
		Sural (31)	Non-remarkable
		<u>Epon</u> Muscular Nerve	Non-remarkable
		Sural Nerve	Non-remarkable
		Lumbar Cord	No changes that can be distinguished from artifacts.
		Cervical Cord	No changes that can be distinguished from artifacts.
	AC5440	<u>Teased Nerve Fibers (No.)</u> Muscular (32)	Non-remarkable
		Sural (31)	Non-remarkable
		<u>Epon</u> Muscular Nerve	Non-remarkable
		Sural Nerve	Non-remarkable
		Lumbar Cord	No definitive changes
		Cervical Cord	No definitive changes

TABLE K-1: SPECIAL NEUROPATHOLOGIC STUDIES - MALES

90-DAY VAPOR INHALATION TOXICITY STUDY
 IN FISCHER 344 RATS

TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-III	AC5379	<u>Teased Nerve Fibers</u> Muscular (38)	Twenty-three fibers show segmented, paranodal and internodal axonal swelling. Occasional fibers show atrophic-like changes and slight clumping of myelin.
		Sural (32)	Two fibers show segmental, paranodal and internodal axonal swelling.
		<u>Epon</u> Muscular Nerve	A small number of individual fibers show axonal swelling in cross and longitudinal sections.
		Sural Nerve	A minimal number of individual fibers show axonal swelling in cross and longitudinal sections.
		Lumbar Cord	A moderate to low number of individual fibers show axonal swelling in the ventral and lateral funiculi. Questionable axonal swelling in the dorsal funiculi.

TABLE K-1: SPECIAL NEUROPATHOLOGIC STUDIES - MALES
90-DAY VAPOR INHALATION TOXICITY STUDY
IN FISCHER 344 RATS

TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-III (cont.)	AC5379 (cont.)	Cervical Cord	A minimal number of fibers in the ventral and lateral funiculi show axonal swelling. Dorsal funiculi show a minimal to small number of fibers with swollen axons.
	AC5399	<u>Teased Nerve Fibers</u> Muscular (45)	Twelve fibers show definitive segmental axonal swellings, primarily internodal, but is also present in paranodal regions. Five fibers show definitive segmental axonal swelling primarily internodal. Other fibers show questionable axonal swellings.
		Sural (41)	
		<u>Epon</u> Muscular Nerve	Multiple fibers show marked axonal swelling in longitudinal and cross sections. Vacuolative change is present, one (A1). Multiple fibers show axonal swelling in both cross and longitudinal sections.
		Sural Nerve	

TABLE K-1: SPECIAL NEUROPATHOLOGIC STUDIES - MALES

90-DAY VAPOR INHALATION TOXICITY STUDY
IN FISCHER 344 RATS

TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-III (cont.)	AC5399 (cont.)	Lumbar Cord	Mild to moderate axonal swelling of individual fibers in ventral and lateral funiculi, questionable in dorsal funiculi. Most fibers are located near the periphery.
		Cervical Cord	Minimal to mild axonal swelling of individual fibers in ventral and lateral funiculi, questionable in dorsal funiculi.
	AC5409	<u>Teased Nerve Fibers</u> Muscular (31)	Fourteen fibers show both paranodal and internodal segmental axonal swellings. One fiber shows distortion and vacuolation of the myelin sheath in area of swelling.
		Sural (30)	Two fibers show multiple axonal swelling, internodal and paranodal. Three other fibers show questionable axonal swelling.
		<u>Epon</u> Muscular Nerve	Axonal swelling of individual fibers is present to a mild degree in both cross and longitudinal sections.

TABLE K-1: SPECIAL NEUROPATHOLOGIC STUDIES - MALES

90-DAY VAPOR INHALATION TOXICITY STUDY
 IN FISCHER 344 RATS

TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-III (cont.)	AC5409 (cont.)	Sural Nerve	Axonal swelling of individual fibers is present in cross section to a minimal to mild degree. No definitive changes in longitudinal sections.
		Lumbar Cord	Numerous individual fibers in ventral and lateral funiculi show axonal swelling (moderate). Isolated macrophages with debris are present. Dorsal funiculi has questionable axonal swelling.
		Cervical Cord	Individual fibers in all funiculi show axonal swelling that is most concentrated on the periphery. Artifacts are present in the dorsal funiculi.
	AC5432	<u>Teased Nerve Fibers</u> Muscular (33)	Twenty-four fibers show marked, segmental paranodal and internodal axonal swelling. A small number of affected fibers show clumping of myelin with other areas appearing atrophic where myelin is no longer distinguishable.

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TABLE K-1: SPECIAL NEUROPATHOLOGIC STUDIES - MALES
90-DAY VAPOR INHALATION TOXICITY STUDY
IN FISCHER 344 RATS

TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-III (cont.)	AC5432 (cont.)	Sural (33)	Five fibers show mild, segmental, paranodal, and internodal axonal swellings.
		<u>Epon</u> Muscular Nerve	A moderate number of individual fibers show axonal swelling on both longitudinal and cross sections. One cross section contains a macrophage with myelin debris.
		Sural Nerve	A minimal number of individual fibers show axonal swelling on longitudinal and cross sections.
		Lumbar Cord	A moderate number of individual fibers show axonal swelling in the ventral and lateral funiculi - most severe in ventral region and on the periphery - one axon shows vacuolative degeneration. Questionable changes are present in dorsal funiculi.
		Cervical Cord	A minimal to mild number of individual fibers show axonal swelling that is most marked in ventral and lateral funiculi.

TABLE K-1: SPECIAL NEUROPATHOLOGIC STUDIES - MALES

**90-DAY VAPOR INHALATION TOXICITY STUDY
 IN FISCHER 344 RATS**

TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-III (cont.)	AC5434	<u>Teased Nerve Fibers</u> <u>Muscular Nerve (40)</u>	Sixteen fibers show segmental axonal swelling in paranodal and internodal areas. Two additional fibers show degenerative-like loss of myelin sheath with clumps of myelin remaining in some areas.
		Sural Nerve (33)	One fiber shows segmental, internodal, paranodal axonal swelling.
		<u>Epon</u> <u>Muscular Nerve</u>	Cross sections show a mild number of fibers with axonal swelling. Longitudinal sections show a minimal number of fibers with axonal swelling.
		Sural Nerve	Cross and longitudinal sections show a minimal number of fibers with axonal swellings.
		Lumbar Cord	A mild to moderate number of fibers in the ventral and lateral funiculi show axonal swelling. One axon appears degenerative. Questionable axonal swelling is present in the dorsal funiculi.

TABLE K-1: SPECIAL NEUROPATHOLOGIC STUDIES - MALES

90-DAY VAPOR INHALATION TOXICITY STUDY
IN FISCHER 344 RATS

TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-III (cont.)	AC5434 (cont.)	Cervical Cord	A minimal number of fibers in the ventral and lateral funiculi show axonal swelling. One macrophage is visible. The dorsal funiculi show a small number of fibers with axonal swelling.

TABLE K-2: SPECIAL NEUROPATHOLOGIC STUDIES - FEMALES

**90-DAY VAPOR INHALATION TOXICITY STUDY
 IN FISCHER 344 RATS**

TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
Untreated Control	AC5465	<u>Teased Nerve Fibers</u> Muscular (32) Sural (29)	Non-remarkable Non-remarkable
		<u>Epon</u> Muscular Nerve Sural Nerve Lumbar Cord Cervical Cord	Non-remarkable Non-remarkable Some variation in the size of axons particularly in the interior white tracts - probably fixation Some variation in the size of axons particularly in the interior white tracts - probably fixation
	AC5478	<u>Teased Nerve Fibers</u> Muscular (38) Sural (32)	Non-remarkable Non-remarkable
		<u>Epon</u> Muscular Nerve Sural Nerve Lumbar Cord Cervical Cord	Non-remarkable Non-remarkable No changes that can be distinguished from artifacts No changes that can be distinguished from artifacts
	AC5486	<u>Teased Nerve Fibers</u> Muscular (30) Sural (34) (Slide #2)	Non-remarkable Non-remarkable
		<u>Epon</u> Muscular Nerve Sural Nerve Lumbar Cord Cervical Cord	Non-remarkable Non-remarkable Non-remarkable Non-remarkable

TABLE K-2: SPECIAL NEUROPATHOLOGIC STUDIES - FEMALES

90-DAY VAPOR INHALATION TOXICITY STUDY
 IN FISCHER 344 RATS

TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
Untreated Control (cont.)	AC5487	<u>Teased Nerve Fibers</u>	
		Muscular (41)	Non-remarkable
		Sural (31)	Non-remarkable
		<u>Epon</u>	
		Muscular Nerve	Non-remarkable
		Sural Nerve	Non-remarkable
		Lumbar Cord	Two fibers in the lateral funiculi show distended myelin sheath and do not contain axonal material.
		Cervical Cord	Non-remarkable
	AC5530	<u>Teased Nerve Fibers</u>	
		Muscular (32)	Non-remarkable
Sural (29)		Non-remarkable	
<u>Epon</u>			
		Muscular Nerve	Non-remarkable
		Sural Nerve	Non-remarkable
	Lumbar Cord	Non-remarkable	
	Cervical Cord	Non-remarkable	

TABLE K-2: SPECIAL NEUROPATHOLOGIC STUDIES - FEMALES
 90-DAY VAPOR INHALATION TOXICITY STUDY
 IN FISCHER 344 RATS

TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings	
T-II	AC5467	<u>Teased Nerve Fibers (No.)</u>		
		Muscular (31)	Non-remarkable	
		Sural (34)	One fiber shows equivocal unilateral axonal swelling at the node - may be an artifactual change.	
		<u>Epon</u>		
		Muscular Nerve	Three fibers in cross section, C-2, show equivocal axonal swelling.	
		Sural Nerve	Non-remarkable	
	AC5468	AC5468	Lumbar Cord	Non-remarkable
			Cervical Cord	Non-remarkable
			<u>Teased Nerve Fibers (No.)</u>	
			Muscular (32)	Non-remarkable
			Sural (31)	Non-remarkable
			<u>Epon</u>	
AC5485	AC5485	Muscular Nerve	No definitive changes	
		Sural Nerve	No definitive changes	
		Lumbar Cord	No definitive changes	
		Cervical Cord	No definitive changes	
		<u>Teased Nerve Fibers (No.)</u>		
		Muscular (32)	No definitive lesions	
AC5494	AC5494	Sural (26)	No definitive lesions	
		<u>Epon</u>		
		Muscular Nerve	No definitive lesions	
		Sural Nerve	No definitive lesions	
		Lumbar Cord	No definitive lesions	
		Cervical Cord	No definitive lesions	
AC5494	AC5494	<u>Teased Nerve Fibers (No.)</u>		
		Muscular (30)	No definitive lesions. Questionable bilateral swelling of one fiber.	
		Sural (33)	No definitive lesions	

TABLE K-2: SPECIAL NEUROPATHOLOGIC STUDIES - FEMALES
 90-DAY VAPOR INHALATION TOXICITY STUDY
 IN FISCHER 344 RATS

TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings	
T-II (cont.)	AC5494 (cont.)	<u>Epon</u> Muscular Nerve	One fiber on cross section shows axonal swelling with thinning and disruption of the myelin sheath, slide C-2.	
		Sural Nerve	Non-remarkable	
		Lumbar Cord	Non-remarkable	
		Cervical Cord	Non-remarkable	
	AC5507	<u>Teased Nerve Fibers (No.)</u>		
		Muscular (31)		No definitive lesions
		Sural (32)		No definitive lesions
		<u>Epon</u> Muscular Nerve		Non-remarkable
		Sural Nerve		Non-remarkable
		Lumbar Cord		Non-remarkable
		Cervical Cord	Non-remarkable	

TABLE K-2: SPECIAL NEUROPATHOLOGIC STUDIES - FEMALES

90-DAY VAPOR INHALATION TOXICITY STUDY
IN FISCHER 344 RATS

TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-III	AC5459	<u>Teased Nerve Fibers</u> Muscular (31)	Twenty fibers show axonal swelling and some show clumping of myelin and appear atrophic.
		Sural (31)	Three fibers show axonal swelling primarily unilaterally at the nodes.
		<u>Epon</u> Muscular Nerve	Cross sections show numerous fibers with axonal swelling. Longitudinal sections show small numbers of fibers with swollen axons that are confined to one area. No clear-cut changes.
		Sural Nerve	There is a suggestion of axonal swelling in some fibers.
		Lumbar Cord	Numerous fibers in the ventral and lateral funiculi exhibit axonal swelling. One axon is degenerative. Questionable swollen axons are present in the dorsal funiculus.
		Cervical Cord	Small number of fibers with swollen axons are present in all funiculi.

TABLE K-2: SPECIAL NEUROPATHOLOGIC STUDIES - FEMALES
 90-DAY VAPOR INHALATION TOXICITY STUDY
 IN FISCHER 344 RATS

TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-III (cont.)	AC5472	<u>Teased Nerve Fibers</u> Muscular (44)	Twenty-eight fibers show axonal swellings and several fibers show clumping of myelin sheaths.
		Sural (32)	Two fibers show minimal paranodal and internodal axonal swelling.
		<u>Epon</u> Muscular Nerve	Numerous fibers show axonal swelling of both cross and longitudinal sections.
		Sural Nerve	Small number of fibers in cross section show axonal swelling. No definitive change in the longitudinal section.
		Lumbar Cord	Numerous fibers in the ventral and lateral funiculi show axonal swelling. Isolated macrophages are present. Questionable axonal swelling is present in the dorsal funiculi.
		Cervical Cord	A minimal of fibers with swollen axons are present in all funiculi.

TABLE K-2: SPECIAL NEUROPATHOLOGIC STUDIES - FEMALES

90-DAY VAPOR INHALATION TOXICITY STUDY
IN FISCHER 344 RATS

TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-III (cont.)	AC5523	<u>Teased Nerve Fibers</u> Muscular (28)	Eleven fibers show axonal swelling of a mild to moderate degree at the internodal and nodal areas in a segmented pattern.
		Sural (33)	Twenty-one nerves show segmental axonal swelling at paranodal and internodal regions.
		<u>Epon</u> Muscular Nerve	Longitudinal sections show numerous fibers with swollen axons. The nerve appears edematous and one macrophage containing debris is present. Also there are areas of remyelination - questionable type changes.
		Sural Nerve	Cross and longitudinal sections show small numbers of fibers with swollen axons.
		<u>Epon</u> Lumbar Cord	The lateral and ventral funiculi show numerous swollen axons with isolated macrophages containing myelin debris. Dorsal funiculi show small numbers of questionable swollen axons.

ToxiGenics

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TABLE K-2: SPECIAL NEUROPATHOLOGIC STUDIES - FEMALES
90-DAY VAPOR INHALATION TOXICITY STUDY
IN FISCHER 344 RATS

TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-III (cont.)	AC5523 (cont.)	Cervical Cord	A small number of swollen axons are present in the ventral, lateral, and dorsal funiculi.
	AC5524	<u>Teased Nerve Fibers</u> Muscular (30)	Seven fibers show mild to moderate paranodal and internodal axonal swelling.
		Sural (29)	No definitive changes.
		<u>Epon</u> Muscular Nerve	A moderate number of fibers show axonal swelling on cross sections and a small number on longitudinal sections. Areas suggestive of remyelination are present. Also a macrophage is located in the interstitium.
		Sural Nerve	Small numbers of fibers in both longitudinal and cross sections show swollen axons.
		Lumbar Cord	Numerous fibers in the ventral and lateral funiculi show swollen axons with an occasional axon showing vacuolation. Dorsal funiculi contains questionable swollen axons.

TABLE K-2: SPECIAL NEUROPATHOLOGIC STUDIES - FEMALES

90-DAY VAPOR INHALATION TOXICITY STUDY
 IN FISCHER 344 RATS

TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-III (cont.)	AC5524 (cont.)	Cervical Cord	All funiculi contain small numbers of fibers with swollen axons. A macrophage with myelin is present in the dorsal funiculi.
	AC5531	<u>Teased Nerve Fibers</u> Muscular (35)	Twelve fibers show axonal swelling in paranodal and internodal areas. An additional five fibers show clumping of myelin and atrophic areas with significant axonal swelling.
		Sural	No definitive change, one fiber shows a suggestive unilateral nodal swelling.
		<u>Epon</u> Muscular Nerve	A moderate number of fibers show axonal swelling in both longitudinal and cross sections.
		Sural Nerve	A minimal number of fibers show axonal swelling; and on longitudinal sections, they appear primarily paranodal, unilaterally.

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TABLE K-2: SPECIAL NEUROPATHOLOGIC STUDIES - FEMALES

**90-DAY VAPOR INHALATION TOXICITY STUDY
IN FISCHER 344 RATS**

TEST ARTICLE: CARBON DISULFIDE

Group	Animal Number	Specimen	Findings
T-III (cont.)	AC5531 (cont.)	Lumbar Cord	Moderately severe axonal swelling is present in the ventral and lateral funiculi. An isolated macrophage is present. The dorsal funiculi shows minimal numbers of fibers with swollen axons.
		Cervical Cord	A minimal number of fibers with swollen axons are present in all funiculi.

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Appendix B

Study 420-0711A (Fischer 344 Rats)

Detailed Neuropathological Findings

T-I Treatment Group (50 ppm)

Test Article: Carbon Disulfide

Study 420-0711A (Fischer 344 Rats)

90-Day Vapor Inhalation Toxicity Study in Fischer 344 Rats

Detailed Neuropathological Findings

T-I Treatment Group (50 ppm)

Test Article: Carbon Disulfide

Animal Number	Sex	Teased Fiber Study		Epon Sections			
		Muscular N.	Sural N.	Muscular N.	Sural N.	Lumbar Cord	Cervical Cord
AC5392	M	0	0	0	0	0	0
AC5397	M	0	0	0	0	0	0
AC5406	M	0	0	0	0	0	0
AC5444	M	0	0	0	0	0	0
AC5445	M	0	0	0	0	0	0
AC5500	F	0	0	0	0	0	0
AC5503	F	0	0	0	0	0	0
AC5511	F	0	0	0	0	0	0
AC5537	F	0	0	0	0	0	0
AC5538	F	0	0	0	0	0	0

Teased nerve fibers and epon sections were examined for evidence of axonal swellings or degeneration. Lesions were absent from this treatment group.

Appendix C

Quality Assurance Statement

Quality Assurance Inspection and Reporting Dates

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CHEMICAL INDUSTRY INSTITUTE OF TOXICOLOGY
6 Davis Drive, P.O. Box 12137
Research Triangle Park, NC 27709

QUALITY ASSURANCE STATEMENT

STUDY TITLE: Addendum to 90-Day Vapor Inhalation Toxicity Study of Carbon Disulfide in Fischer 344 Rats; Subject: Special Neuropathological Examination (ToxiGenics, Inc. Study No. 420-0711A)

The conduct of this study was subjected at ToxiGenics, Inc. and Microbiological Associates to periodic Quality Assurance inspections. The dates of inspections and the dates that findings were reported to the Study Director and Management were included in CIIT Docket 52063, page 4 (Vol. I) and page 153 (Vol. III), respectively.

This addendum report has been reviewed by Chemical Industry Institute of Toxicology's Quality Assurance in accordance with FDA's Good Laboratory Practice Regulation, CFR 21 Part 58. The dates of inspection and the dates that findings were reported to the Study Director/Management are attached to this report.

Patricia O'Brien Pomerleau
Patricia O'Brien Pomerleau, M.S.
Quality Assurance Manager

07/05/92
Date

CHEMICAL INDUSTRY INSTITUTE OF TOXICOLOGY
6 Davis Drive, P.O. Box 12137
Research Triangle Park, NC 27709

QUALITY ASSURANCE INSPECTION AND REPORTING DATES

Study Title: Addendum to 90-Day Vapor Inhalation Toxicity Study of Carbon Disulfide in Fischer 344 Rats; Subject: Special Neuropathological Examination (ToxiGenics, Inc. Study No. 420-0711A)

Dates of
Inspection

09/17/91
01/20/92,
02/24/92,
02/27-28/92,
03/02/92,
‡
03/17&20/92
07/08/92

Dates of Report
to Study Director/Management

09/19/91
03/23/92
07/08/92