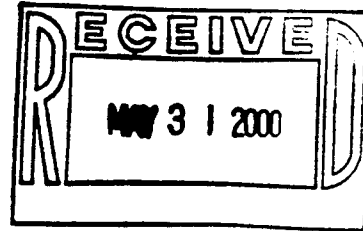




May 30, 2000

Dr. Andrew Seacat
3M Corporate Toxicology
Building 220-2E-02
PO Box 33220
St. Paul, MN 55133-3220



Re: Draft Electron Microscopy Report, Study No. 6329-223 (Recovery Animals)

Dear Dr. Seacat,

Please find enclosed the draft electron microscopy report for the recovery sacrifice animals on Covance Study No. 6329-223. Light and ultrastructural findings identified in the liver of monkeys after 26 weeks of treatment have disappeared in the tissues from recovery sacrifice animals, indicating resolution of the lesions.

After your review and approval of this draft report, I will prepare a signed final report. Please retain the glossy report figures and all glossy electron micrographs for insertion into the final report, as these are originals.

It has been a pleasure working with 3M and Covance on this study. I hope that you will provide PAI with opportunities to work with you again in the future.

Sincerely,

James B. Nold, DVM, PhD
Vice President, Southeast Operations

Enclosure: Draft electron microscopy report, 6329-223



**DRAFT PATHOLOGY REPORT
(ANCILLARY STUDY)**

**ELECTRON MICROSCOPIC EVALUATION
OF LIVER IN CYNOMOLGUS MONKEYS
(RECOVERY ANIMALS)**

**26-WEEK CAPSULE TOXICITY STUDY WITH PERFLUOROOCTANE
SULFONIC ACID POTASSIUM SALT (PFOS;T-6295) IN CYNOMOLGUS
MONKEYS**

**CLIENT STUDY NUMBER 6329-223
PAI STUDY NUMBER EM 99.76**

Prepared by:

James B. Nold, D.V.M., Ph.D., Diplomate, A.C.V.P.

**Pathology Associates International
4915-D Prospectus Drive
Durham, NC 27713**

May 30, 2000

**PREPARED FOR 3M CORPORATE TOXICOLOGY,
3M CENTER, ST. PAUL, MN 55144-1000**

	<u>Section</u>
Pathology Narrative	I
Light Microscopy and Transmission Electron Micrograph Interpretation Forms	II
Transmission Electron Micrographs	III
Quality Assurance Statement	IV

I. Pathology Narrative

**DRAFT PATHOLOGY REPORT
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SUMMARY

Two male and two female cynomolgus monkeys treated for 26 weeks with 0.75 mg/kg PFOS were removed from treatment for up to 53 weeks. Liver samples obtained by biopsy at Week 57 and necropsy at Week 79 were examined for reversibility, persistence, or delayed occurrence of toxic effects. Light and electron microscopic evaluation of livers identified no pathological changes. The treatment-related changes of hepatocellular hypertrophy and vacuolation, noted at 26 weeks and characterized ultrastructurally by increased numbers of lipid droplets within hepatocytes, had completely disappeared during the recovery period.

PROCEDURES

The objective of this study was to examine by electron microscopy the livers from selected animals in a 26-week capsule toxicity study with PFOS in cynomolgus monkeys. In addition to a general qualitative evaluation for differences between control and treated animals, the ultrastructural endpoints included quantitation of peroxisomes within hepatocytes and membrane and mitochondrial changes in hepatocytes. Light microscopy of paraffin-embedded liver sections was also performed.

This report details the light and electron microscopy findings in the four high-dose male and female monkeys designated as recovery animals. A previous report (dated 6-24-1999) indicated that treatment of cynomolgus monkeys with 0.75 mg PFOS/kg/day for 26 weeks resulted in hepatocellular vacuolation and hypertrophy by light microscopy. Hepatocellular vacuolation and hypertrophy were primarily centrilobular in distribution and did vary in incidence and severity between individual animals. Ultrastructurally, there were increased lipid droplets within hepatocytes. Increased lipid droplets were

present in both the high-dose males examined and in two of four high-dose females examined. Increased hepatocellular glycogen was present in the remaining two high-dose females. These ultrastructural changes correlated with hepatocellular hypertrophy and vacuolation noted by light microscopy and with increased liver weight parameters in the high-dose animals. The no-effect level for hepatocellular vacuolation (lipid droplet accumulation) was 0.15 mg/kg/day of PFOS (mid-dose group). Quantitation of peroxisomes within hepatocytes by electron microscopy indicated no remarkable differences between control livers and livers treated with 0.03, 0.15, or 0.75 mg PFOS/kg/day. Ultrastructural differences in cellular membranes or mitochondria were not discernible between control and treated animals in the samples examined. The recovery animals were examined to assess for reversibility or persistence of these hepatocellular changes, or for the delayed occurrence of additional PFOS-induced effects.

For the toxicity study, 22 male and 22 female cynomolgus monkeys were given the test article or the vehicle once daily for at least 26 weeks according to Text Table 1.

Text Table 1. Group Designations and Dosage Levels				
Dosage Group	Dose Level (mg/kg/day) ^a	Total Material Dose Level (mg/kg/day) ^b	Number of Males	Number of Females
I	0 ^a	30 ^a	6 ^d	6 ^d
II	0.03	15 ^b	4	4
III	0.15	6 ^c	6 ^d	6 ^d
IV	0.75	30 ^c	6 ^d	6 ^d

^a Dose levels were provided. The control group (Group 1) received an equivalent amount of lactose in gelatin capsules as the total material administered to Group 4.

^b The low-dose group (Group 2) received the test material diluted with lactose (1:499, w:w).

^c The mid-dose (Group 3) and high-dose (Group 4) groups received the test material diluted with lactose (1:39, w:w).

^d Two animals in Groups 1, 3, and 4, designated as recovery animals, were treated for at least 26 weeks, then treatment was discontinued and the animals were observed for reversibility, persistence, or delayed occurrence of toxic effects for at least 52 weeks posttreatment.

During Week 57 (approximately 31 weeks into the recovery period), the 2 high-dose recovery males and 2 high-dose recovery females were anesthetized and liver biopsies were surgically obtained from each animal and chemically preserved and processed as described below.

During Week 79, the 4 high-dose recovery animals were necropsied and liver samples obtained as described below. The two control recovery animals were returned to the Covance stock animal colony, and the two Group III animals were transferred to a subsequent study (Covance 6329-268). Liver samples were not obtained and examined in the control recovery or Group III recovery animals.

Animals to be sacrificed at recovery sacrifice times were anesthetized with ketamine and xylazine, weighed, exsanguinated, and necropsied. Multiple tissues were collected and preserved in 10% neutral-buffered formalin according to the protocol. Additionally,

pieces of liver from each animal were thin-sliced and placed in ½ strength Karnovsky's fixative (2.0% paraformaldehyde/2.5% glutaraldehyde in 0.1M phosphate buffer) for subsequent ultrastructural examination. These livers were processed into Epon blocks and submitted to this laboratory for subsequent electron microscopy processing and evaluation. Hematoxylin and eosin (H&E)-stained slide sections of formalin-fixed, paraffin-embedded livers were submitted for light microscopic examination.

The four high-dose recovery animals are listed in Text Table 2. For each animal, there was a 57-Week liver biopsy sample and a 79-Week terminal necropsy liver sample.

Text Table 2. Recovery Animals Examined by Light and Electron Microscopy	
0.75 mg/kg/day PFOS (Group 4 High-Dose)	
Males	Females
I05511	I05533
I05522	I05542

Thin sections (approximately 90 nm) from the Epon-embedded livers were cut, mounted on 200-mesh copper grids, stained with 5% methanolic uranyl acetate and Reynold's lead citrate, and examined on a Zeiss 900 transmission electron microscope. Centrilobular hepatocytes, where clearly identifiable in liver sections, were preferentially examined. Representative electron photomicrographs of liver were taken and significant ultrastructural features were summarized for each photograph and animal on a designated transmission electron micrograph interpretation form. The number of peroxisomes in hepatocytes was manually counted for each center photographed hepatocyte and recorded. For light microscopy, the H&E slides from the same animals selected for electron microscopy were evaluated and significant features were summarized on a light microscopic evaluation form.

RESULTS AND DISCUSSION

Individual interpretations of light microscopy slides and electron micrographs for each animal are shown in Section II. Representative electron photomicrographs were selected as report figures, labeled, and are shown in Section III. All remaining electron micrographs are separately attached to this report.

Light Microscopy

A summary of the incidence and severity of light microscopic findings in the control and high-dose livers is shown in Text Table 3. There were no microscopic findings that appeared to be associated with test material administration. Hepatocellular hypertrophy and vacuolation, noted after 26 weeks of treatment in some high-dose animals, were not identified in recovery samples at either the 57-Week biopsy or 79-Week terminal necropsy samples, indicating complete reversal of the light microscopic liver changes.

The liver findings noted in the recovery animals were considered normal background histopathology of cynomolgus monkeys.

Text Table 3. Incidence and Severity of Light Microscopic Liver Findings -- Recovery Animals		
	0.75 mg/kg/day (High-Dose)	
	Males	Females
(number examined)	(2)	(2)
57-Week Biopsy		
Infiltrate, lymphohistiocytic, minimal	1	2
Terminal Necropsy		
Infiltrate, lymphohistiocytic, minimal	2	2
Hyperplasia, bile duct, slight	1	—
Inflammation, lymphohistiocytic, slight	1	—
Inflammation, eosinophilic, slight	1	—
Clearing, cytoplasmic, centrilobular, minimal	—	1

— Incidence of zero.

Electron Microscopy

Electron microscopic evaluation of the 57-week biopsy and 79-week necropsy liver samples identified no abnormal features, and all tissue samples were considered to be ultrastructurally normal (Text Table 4). The hepatocellular hypertrophy and vacuolation seen after 26 weeks of treatment, and characterized ultrastructurally by increased lipid droplets, were not seen in the recovery animal liver samples, indicating recovery of these test material related effects. The electron microscopic features described in these recovery samples were considered normal ultrastructural findings in hepatocytes. Figures 1-4 are representative electron micrographs from these samples and illustrate normal liver and hepatocellular features.

Text Table 4. Summary of Ultrastructural Findings – Recovery Animals		
Treatment Group (mg/kg/day)/Sex	Animal No.	Comments
(0.75) Male, 57-week biopsy	I05511	Normal
(0.75) Male, 79- week necropsy	I05511	Normal
(0.75) Male, 57-week biopsy	I05522	Normal
(0.75) Male, 79-week necropsy	I05522	Normal
(0.75) Female, 57-week biopsy	I05533	Normal
(0.75) Female, 79-week necropsy	I05533	Normal
(0.75) Female, 57-week biopsy	I05542	Normal
(0.75) Female, 79-week necropsy	I05542	Normal

Peroxisomes were identified and quantitated in representative hepatocytes (Text Table 5). Mean peroxisome numbers per hepatocyte were considered normal.

Text Table 5. Quantitation of Hepatocellular Peroxisomes							
0.75 mg PFOS/kg/day (High-Dose) Recovery Animals							
Male				Female			
57-week biopsy		79-week necropsy		57-week biopsy		79-week necropsy	
Animal Number	Number ¹ of Peroxisomes	Animal Number	Number of Peroxisomes	Animal Number	Number of Peroxisomes	Animal Number	Number of Peroxisomes
I05511	18.5	I05511	19.2	I05533	29.8	I05533	20.8
I05522	19.4	I05522	25.8	I05542	23.0	I05542	15.2
Mean =	18.95	Mean =	22.5	Mean =	26.4	Mean =	18.0

¹ Average number of peroxisomes per hepatocyte for five hepatocytes counted per animal. For Animal No. I05511 (biopsy), peroxisomes were counted in only four hepatocytes

CONCLUSION

Light and electron microscopic evaluation of livers from cynomolgus monkeys treated with 0.75 mg PFOS/kg/day for 26 weeks and allowed to "recover" untreated for 31 and 53 weeks identified no pathological changes. The treatment-related changes of hepatocellular hypertrophy and vacuolation, characterized ultrastructurally by increased numbers of lipid droplets within hepatocytes at 26 weeks, had completely resolved during the recovery period.

SIGNATURE OF AUTHOR

James B. Nold, D.V.M., Ph.D.,
Diplomate, A.C.V.P.

Date

II. Light Microscopy and Transmission Electron Micrograph Interpretation Forms ¹

¹ Forms are exact copies of signed/dated raw data sheets maintained in archived study file.

LIGHT MICROSCOPIC EVALUATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkeys

Animal No: see below

Tissue: Liver

Sex: see below

Interpreting Pathologist (signature and date): James B. Nord April 7, 2000

Dose/Group	Animal Number	Microscopic Findings and Comments
0.75 mg/kg (High-dose) Recovery Males 57 wk biopsy	I05511	1. Infiltrate, lymphohistiocytic, multifocal, minimal
	I05522	Normal
0.75 mg/kg (High-dose) Recovery Females 57 wk biopsy	I05533	1. Infiltrate, lymphohistiocytic, focal, minimal
	I05542	1. Infiltrate, lymphohistiocytic, multifocal, minimal
0.75 mg/kg (High-dose) Recovery Males 79 wk necropsy	I05511	1. Infiltrate, lymphohistiocytic, multifocal, minimal
	I05522	1. Infiltrate, lymphohistiocytic, multifocal, minimal 2. Hyperplasia, focal, slight, bile duct 3. Inflammation, lymphohistiocytic, multifocal, slight 4. Inflammation, eosinophilic, multifocal, slight
0.75 mg/kg (High-dose) Recovery Females 798 week necropsy	I05533	1. Infiltrate, lymphohistiocytic, multifocal, minimal
	I05542	1. Infiltrate, lymphohistiocytic, multifocal, minimal 2. Clearing, cytoplasmic, centrilobular, minimal

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05511

Tissue: Liver - centrilobular

57-week biopsy
Treatment Group: .75 mg/kg (hi-dose)

Block No(s): 99.76-31-1A
Z18823 -

Sex: Male

Photo/Negative No(s): Z18827

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): James B. N. et al May 26, 2000

Features: Z18823. Liver. Hepatocyte. 15,290X. This hepatocyte has a small round central nucleus. The surrounding cytoplasm contains numerous round to oval mitochondria and several small to large lipid droplets. The darker round organelles, mostly slightly smaller than the mitochondria, are peroxisomes. Twenty-four (24) peroxisomes counted. The intervening cytosol contains profiles of endoplasmic reticulum and some fine granules, which may be glycogen.

Z18824. Liver. 10,320X. Portions of three cells are present. The dorsal two hepatocytes exhibit a mixture of normal appearing cytoplasmic elements, including mitochondria, endoplasmic reticulum and scattered peroxisomes. The larger ventral cell's cytoplasm contains irregular clear spaces surrounded by membranous material. Only a couple of mitochondria are evident, and this cell is probably not a hepatocyte. Around the periphery are several large structures containing finely granular and reticular material. This may represent smooth endoplasmic reticulum intermixed with glycogen and/or ribosomes. It appears to be membrane bound, but this may be a pseudomembrane caused by compacting of the surrounding cytoplasm. Or, these structures may represent cytoplasmic invaginations of extracellular material such as plasma. The nuclear profiles in these cells appear normal. Peroxisomes were not counted in this electron micrograph because an individual hepatocyte showing most of its cytoplasm is not present.

Z18825. Liver. Hepatocyte. 10,320X. This hepatocyte is polygonal with a round central nucleus. Its surrounding cytoplasm contains numerous mitochondria and a few small lipid droplets. The intervening cytoplasm contains profiles of endoplasmic reticulum, scattered fine glycogen granules, and some dark round peroxisomes. Twenty-two (22) peroxisomes counted. Three adjacent hepatocytes appear essentially normal. At the bottom right of the photograph is a finely granular structure, which is outside of the centered hepatocyte and appears similar to that described in Z18224.

Z18826. Liver. Hepatocyte. 10,320X. This hepatocyte is bordered by an extracellular space along its right margin. The cell has a small nuclear profile and abundant cytoplasm containing numerous mitochondria and slightly dilated endoplasmic reticulum. Several lipid droplets are present. Seventeen (17) peroxisomes counted.

I05511 Biopsy Continued.

Z18827. Liver. Hepatocyte. 10,320X. A large round nucleus is present in the centered hepatocyte. The surrounding cytoplasm contains numerous mitochondria and abundant endoplasmic reticulum. A few dark residual bodies are present. Eleven (11) peroxisomes counted. At the right dorsal margin of the photograph are an endothelial cell and a granular structure that appears to be an invagination of plasma.

Conclusions: Normal hepatocytes. Peroxisomes average 18.5 per hepatocyte.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05511

Tissue: Liver - centrilobular

79-week necropsy
Treatment Group: .75 mg/kg (hi-dose)

Block No(s): 99.76-32-2A
Z18828 -

Sex: Male

Photo/Negative No(s): Z18832

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): *James R. N. Ed* May 29, 2000

Features: Z18828. Liver. Hepatocyte. 10,320X. This hepatocyte is somewhat oval in shape with a small round nucleus. Approximately 8-9 small to medium sized lipid droplets are present in the cytoplasm. The majority of the cytoplasm contains numerous round to rod-shaped mitochondria, intervening areas of smooth endoplasmic reticulum (SER) and "more clear" patches of glycogen. Some profiles of rough endoplasmic reticulum (RER) and round dark peroxisomes are also evident. Twenty-eight (28) peroxisomes counted. Bile canaliculi can be seen between the hepatocytes situated on the right and left with the central hepatocyte.

Z18829. Liver. Hepatocyte. 10,320X. The central vein and surrounding extracellular collagen are present at the top right margin of this electron micrograph. A sinusoid is also present at the lower left margin. The intervening hepatocyte has a round nucleus and surrounding cytoplasm containing numerous profiles of endoplasmic reticulum and dark mitochondria. There is mild dilatation of the endoplasmic reticulum and nuclear envelope, which is a fixation artifact. Several dark residual bodies and occasional small lipid droplets are also identified. Differentiation between peroxisomes and mitochondria is difficult. Eleven (11) peroxisomes counted.

Z18830. Liver. Hepatocyte. 10,320X. The cytoplasm of this hepatocyte contains abundant SER, although some profiles of RER are also evident, especially around some mitochondria. A nuclear profile is not evident in this plane of section of the centered hepatocyte. Approximately 20 lipid droplets are present. Twenty (20) peroxisomes counted. This hepatocyte's microvillous border is evident both dorsally and ventrally along sinusoids. The nucleus of an endothelial cell is present at the lower right. Adjacent hepatocytes are present to the left and right of the centered hepatocyte.

Z18831. Liver. Hepatocyte. 10,320X. The morphology of this hepatocyte is very similar to Z18828. The cytoplasm contains numerous mitochondria, endoplasmic reticulum and "more clear" patches of glycogen. Several lipid droplets are present. Nineteen (19) peroxisomes counted. The surrounding hepatocytes appear ultrastructurally similar.

I05511 Necropsy Continued.

Z18832. Liver. Hepatocyte. 10,320X. This hepatocyte appears similar to Z18830. Its cytoplasm contains abundant SER. Other recognizable organelles include some RER, lipid droplets, mitochondria, peroxisomes and residual bodies. Eighteen (18) peroxisomes counted. At the lower right margin of the photograph is a sinusoid containing several erythrocytes. A prominent bile canaliculus is present at the right where three hepatocytes appear to meet. These hepatocytes are considered to be ultrastructurally normal.

Conclusions: Normal hepatocytes. Average 19.2 peroxisomes per hepatocyte.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05522

Tissue: Liver - centrilobular

57-week biopsy

Treatment Group: .75 mg/kg (hi-dose)

Block No(s): 99.76-33-1A

Z18833 -

Sex: Male

Photo/Negative No(s): Z18837

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): *James B. Noel* May 29, 2000

Features: Z18833. Liver. Hepatocyte. 10,320X. A sinusoid containing finely granular plasma is along the right microvillous margin of this hepatocyte. The hepatocyte has an oval nucleus. In the surrounding cytoplasm are several very electron-dense residual bodies. There is mild dilatation of the nuclear envelope and endoplasmic reticulum, which is a fixation artifact. Eighteen (18) peroxisomes counted. Mitochondria are numerous and are variably shaped.

Z18834. Liver. Hepatocyte. 10,320X. This hepatocyte appears ultrastructurally similar to Z 18833. There is mild dilatation of the endoplasmic reticulum and nuclear envelope. The hepatocyte lines a sinusoid, which contains two erythrocytes and a granulated eosinophil. Eight (8) peroxisomes counted.

Z18835. Liver. Hepatocyte. 10,320X. Several small lipid droplets are present in the cytoplasm of this hepatocyte. It has a slightly oval nucleus and abundant cytoplasm which contains numerous mitochondria and profiles of endoplasmic reticulum. There is slight dilatation of the endoplasmic reticulum and nuclear envelope. A distinct myelin figure is evident associated with a lipid droplet to the left of the nucleus. Twenty-two (22) peroxisomes counted.

Z18836. Liver. Hepatocyte. 10,320X. The hepatocyte has a large polygonal nucleus. The cytoplasm contains abundant SER and RER, which exhibit slight dilatation. Several small to large lipid droplets are present. Numerous round dark peroxisomes are evident. Thirty-five (35) peroxisomes counted. Mitochondria are numerous and variably shaped, but appear ultrastructurally normal.

Z18837. Liver. Hepatocyte. 10,320X. A sinusoid is present along the left and lower margins of this electron micrograph. The lighter-staining endothelial cell is present along the lower margin. The centered hepatocyte has an oval nucleus and numerous mitochondria and endoplasmic reticulum in its surrounding cytoplasm. There is mild dilatation of the endoplasmic reticulum and nuclear envelope. Several lipid droplets and residual bodies are also discernible. Fourteen (14) peroxisomes counted.

Conclusions: Normal hepatocytes. Peroxisomes average 19.4 per hepatocyte.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05522

Tissue: Liver - centrilobular

79-week necropsy

Treatment Group: .75 mg/kg (hi-dose)

Block No(s): 99.76-34-2A

Z18838 -

Sex: Male

Photo/Negative No(s): Z18842

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): *James B. N. et al* May 29, 2000

Features: Z18838. Liver. Hepatocyte. 10,320X. This hepatocyte has a large round nucleus. At the very top left margin of the electron micrograph is a segment of sinusoid along its microvillous border. A bile canaliculus is evident at the lower left margin of the photograph, where the centered hepatocyte meets an adjacent hepatocyte. The centered hepatocyte has abundant cytoplasm, which contains numerous profiles of RER and SER. There is slight dilatation of the endoplasmic reticulum. Mitochondria are numerous are round to oval in shape. Approximately twenty-three (23) peroxisomes counted.

Z18839. Liver. Hepatocyte. 10,320X. Two hepatocytes are clearly evident in this electron micrograph. They appear ultrastructurally similar. They exhibit mild dilatation of their endoplasmic reticulum and nuclear envelopes. They have numerous mitochondria and occasional lipid droplets and residual bodies. Peroxisomes are usually round and more electron-dense than the mitochondria. Seventeen (17) peroxisomes counted in the upper hepatocyte.

Z18840. Liver. Hepatocyte. 10,320X. This hepatocyte has a large round central nucleus. The surrounding cytoplasm has numerous mitochondria and many slightly dilated profiles of endoplasmic reticulum. Both the SER and RER appear slightly dilated. Approximately 15-16 small to medium sized lipid droplets are present, as are a few very electron dense residual bodies. Twenty-one (21) peroxisomes counted.

Z18841. Liver. Hepatocyte. 10,320X. This hepatocyte's nucleus is not evident in this plane of section. A sinusoid containing an erythrocyte is present along the very top margin of this electron micrograph. There is slight dilatation of the endoplasmic reticulum throughout the cytoplasm. Also, random small to medium sized lipid droplets are present. Numerous peroxisomes are evident. Forty (40) peroxisomes counted.

Z18842. Liver. Hepatocyte. 10,320X. This hepatocyte appears similar to Z18838, and is ultrastructurally normal. It has an oval nucleus and abundant surrounding cytoplasm containing numerous mitochondria and slightly dilated profiles of endoplasmic reticulum. Twenty-eight (28) peroxisomes counted. Sinusoids are evident at the upper left and lower right margins of the photograph.

Conclusions: Normal hepatocytes. Peroxisomes average 25.8 per hepatocyte.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05533

Tissue: Liver - centrilobular

57-week biopsy

Treatment Group: .75 mg/kg (hi-dose)

Block No(s): 99.76-35-1A

Z18843 -

Sex: Female

Photo/Negative No(s): Z18847

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): *James B. N. May* May 29, 2000

Features: Z18843. Liver. Hepatocyte. 10,320X. This hepatocyte has a small nuclear profile. A sinusoid lined by an endothelial cell is present along the upper right margin of the electron micrograph, and an adjacent hepatocyte is present at the lower left margin. A prominent bile canaliculus is present between the two hepatocytes. The cytoplasm of the centered hepatocyte is voluminous and contains numerous mitochondria and endoplasmic reticulum. The endoplasmic reticulum, nuclear envelope, and mitochondria appear to be slightly dilated. This dilatation is a fixation artifact. A few small to medium sized lipid droplets are present. Forty (40) peroxisomes counted.

Z18844. Liver. Hepatocyte. 10,320X. This somewhat triangular-shaped hepatocyte has a large oval nucleus. Its cytoplasm contains numerous mitochondria and slightly dilated profiles of RER. Some glycogen granules appear to be intermixed with the SER. Eighteen (18) peroxisomes counted.

Z18845. Liver. Hepatocyte. 10,320X. This electron micrograph appears similar to Z18844, although two nuclear profiles are present, which is probably a function of the plane of section although it may be binucleated. Thirty-four (34) peroxisomes counted.

Z18846. Liver. Hepatocyte. 10,320X. The centered hepatocyte contains a small round nucleus. The surrounding cytoplasm contains numerous mitochondria and slightly dilated profiles of endoplasmic reticulum, both SER and RER. One myelin figure and several lipid droplets are present. Thirty-three (33) peroxisomes counted. Some extracellular collagen and a sinusoid are present along the lower right margin of the photograph. The hepatocyte at the top right is more lightly staining than the centered hepatocyte, and its cytoplasm contain abundant SER. Some mitochondria and peroxisomes are also evident.

Z18847. Liver. Hepatocyte. 10,320X. This electron micrograph appears very similar to Z18844. Although there is slight dilatation of the endoplasmic reticulum, this hepatocyte appears ultrastructurally normal. Twenty-four (24) peroxisomes counted.

Conclusions: Normal hepatocytes. Peroxisomes average 29.8 per hepatocyte.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05533

Tissue: Liver - centrilobular

79-week necropsy

Treatment Group: .75 mg/kg (hi-dose)

Block No(s): 99.76-36-2A

Z18848 -

Sex: Female

Photo/Negative No(s): Z18852

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): *James B. Nelson* May 29, 2000

Features: Z18848. Liver. Hepatocyte. 10,320X. This electron micrograph illustrates a hepatocyte with adjacent hepatocytes along either side. Sinusoids are present along the top right and bottom left margins. Along the sinusoids, the hepatocytes exhibit microvillous borders. Lightly staining endothelial cells line the sinusoids. The centered hepatocyte has a large oval nucleus and abundant surrounding cytoplasm. The cytoplasm contains large amounts of SER and numerous scattered mitochondria. A few lipid droplets and residual bodies are present. RER is evident as clusters, usually around mitochondria. Thirty-five (35) peroxisomes counted.

Z18849. Liver. Hepatocyte. 10,320X. This pentagonally-shaped hepatocyte has a somewhat "trapezoid" appearance and is bounded by hepatocytes on five sides. It has a round central nucleus and abundant surrounding cytoplasm. The cytoplasm contains numerous mitochondria and lots of SER. Some profiles of RER are present around the mitochondria. Twenty-four (24) peroxisomes counted.

Z18850. Liver. Hepatocyte. 10,320X. This hepatocyte is bordered by a sinusoid at its lower left margin. The sinusoid is lined by an endothelial cell and contains two profiles of erythrocytes. The hepatocyte has a small central nucleus. Its cytoplasm contains numerous areas of glycogen. These are lightly stained areas with fine granular material. Mitochondria, SER and RER appear ultrastructurally normal. Eight (8) peroxisomes counted.

Z18851. Liver. Hepatocyte. 10,320X. This electron micrograph appears essentially similar to Z18850. Near the top center there is a sinusoid lined by an endothelial cell and containing an erythrocyte. The hepatocyte's cytoplasm contains multiple patches of glycogen interspersed between SER and mitochondria. Eighteen (18) peroxisomes counted.

Z18852. Liver. Hepatocyte. 10,320X. This hepatocyte is round, with a round nucleus, and contains one medium sized lipid droplet in its cytoplasm. A sinusoid containing an erythrocyte is present along the lower right margin of the electron micrograph. Abundant SER, mitochondria, and some glycogen are present in the cytoplasm of the hepatocyte. Approximately nineteen (19) peroxisomes counted.

Conclusions: Normal hepatocytes. Peroxisomes average 20.8 per hepatocyte.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05542

Tissue: Liver - periportal

57-week biopsy

Treatment Group: .75 mg/kg/ (hi-dose)

Block No(s): 99.76-37-1A

Z18853 -

Sex: Female

Photo/Negative No(s): Z18857

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): *James E. N. Li* May 29, 2000

Features: Z18853. Liver. Hepatocyte. 10,320X. This hepatocyte appears surrounded by adjacent hepatocytes on all sides. It has a large polygonal nucleus and abundant surrounding cytoplasm. Just to the lower left of its nucleus is a small segment of extracellular space containing some microvilli and collagen, probably next to a sinusoid. Several small lipid droplets are present in the cytoplasm of the centered hepatocyte. The endoplasmic reticulum is slightly dilated, and several residual bodies are present. Nineteen (19) peroxisomes counted.

Z18854. Liver. Hepatocyte. 10,320X. Similar to Z18853, the endoplasmic reticulum of this hepatocyte is slightly dilated, which along with the dilated nuclear envelope, is an artifact of fixation. The mitochondria appear to also exhibit some slight artifactual swelling. Multiple bile canaliculi are evident along the margins of this cell's contact with adjacent hepatocytes. Approximately thirteen (13) peroxisomes counted.

Z18855. Liver. Hepatocyte. 10,320X. This hepatocyte has a large round nucleus. It's surrounding cytoplasm contains numerous profiles of SER, which are minimally dilated. Approximately 20 small to medium sized lipid droplets are present in the cytoplasm. Fifty-one (51) peroxisomes counted.

Z18856. Liver. Hepatocyte. 10,320X. Extracellular space containing numerous collagen fibrils is present next to the centered hepatocyte. The hepatocyte has a large oval nucleus and abundant surrounding cytoplasm with numerous mitochondria and endoplasmic reticulum. A few small lipid droplets are present. Thirteen (13) peroxisomes counted.

Z18837. Liver. Hepatocyte. 10,320X. Smooth endoplasmic reticulum and mitochondria are abundant in the cytoplasm of this hepatocyte. The hepatocyte has a large round nucleus and several small to medium sized lipid droplets are present. Nineteen (19) peroxisomes counted.

Conclusions: Normal hepatocytes. Peroxisomes average 23.0 per hepatocyte.

TRANSMISSION ELECTRON MICROGRAPH INTERPRETATION

Study No.: 6329-223 (EM 99.76)

Species/Strain: Cynomolgus Monkey

Animal No.: I05542

Tissue: Liver - centrilobular

79-week necropsy

Treatment Group: .75 mg/kg (hi-dose)

Block No(s): 99.76-38-2A

Z18858 -

Sex: Female

Photo/Negative No(s): Z18862

Significant Lesions (check one): Yes X No

Interpreting Pathologist (signature and date): *James B. N. M.* May 29, 2000

Features: Z18858. Liver. Hepatocyte. 10,320X. This hepatocyte has a round central nucleus. To the left of and beneath the nucleus are several medium sized lipid droplets. The remaining cytoplasm contains abundant SER, patches of glycogen, mitochondria, and some profiles of RER. Six (6) peroxisomes counted.

Z18859. Liver. Hepatocyte. 10,320X. The cytoplasm of this hepatocyte contains multiple areas of glycogen granule accumulation. These are lightly stained areas with numerous small electron dense granules. A few lipid droplets are also present in the cytoplasm. The darker and irregular cytoplasmic material is SER intermixed with glycogen. Five (5) peroxisomes counted.

Z18860. Liver. Hepatocyte. 10,320X. Thirty-two (32) peroxisomes are counted in this hepatocyte's cytoplasm. The cell has a large round nucleus. Most of the cytoplasm is occupied by SER, mitochondria, and occasional lipid droplets. Some profiles of RER are evident around mitochondria. A microvillous border and adjacent sinusoid are present along the right margin of this hepatocyte.

Z18861. Liver. Hepatocyte. 10,320X. For this hepatocyte, its nuclear profile is not present in this plane of section. A moderate number of medium sized lipid droplets are present. Several of these contain some membranous or myelin-like debris. Numerous profiles of SER and RER are present. There is slight dilatation of the endoplasmic reticulum. Numerous variably shaped mitochondria are present, but they appear ultrastructurally normal. Eleven (11) peroxisomes counted.

Z18862. Liver. Hepatocyte. 10,320X. This hepatocyte appears somewhat triangular shaped in this plane of section. It has a large round nucleus and abundant surrounding cytoplasm. Several small to medium sized lipid droplets are present. Abundant SER, some profiles of RER, mitochondria, and peroxisomes occupy the majority of the cytoplasm. Approximately twenty-two (22) peroxisomes counted. A sinusoid with an endothelial cell and erythrocyte is present at the lower left margin of the photograph.

Conclusions: Normal hepatocytes. Peroxisomes average 15.2 per hepatocyte.

III. Transmission Electron Micrographs

Figure Legends

Magnification. Print magnification is indicated on the back of each photograph. The measurement bar on each photomicrograph equals one micron (0.001mm).

Figure 1. Liver. Hepatocyte showing normal cytoplasmic organelles. Some peroxisomes and other normal organelles are identified. There is slight artifactual dilatation of the nuclear envelope and endoplasmic reticulum. Animal No. I05522, 0.75 mg/kg/day (high-dose male), 57-week biopsy, 10,320X magnification.

Figure 2. Liver. Hepatocyte. Glycogen accumulations are shown. Glycogen are the small granules within the clear areas. Animal No. I05511, 0.75 mg/kg/day (high-dose male), 79-week necropsy, 10,320X magnification.

Figure 3. Liver. Hepatocyte showing normal cytoplasmic organelles. There is slight artifactual dilatation of the endoplasmic reticulum and nuclear envelope. Animal No. I05542, 0.75 mg/kg/day (high-dose female), 57-week biopsy, 10,320X magnification.

Figure 4. Liver. Hepatocyte showing normal cytoplasmic organelles. Animal No. I05533, 0.75 mg/kg/day (high-dose female), 79-week necropsy, 10,320X magnification.

Study: 6329-223

PAI EM No.: 99.76-33-1A

Test Article No.: IO5522 Biopsy

Group/Sex: 4-M

Negative No.: Z18835

Magnification: 10,320x

Date: 5/22/00 Tech: NW

Pathology Associates International

4915-D Prospectus Dr.

Durham, NC 27713

(919) 544-5257

Z18835

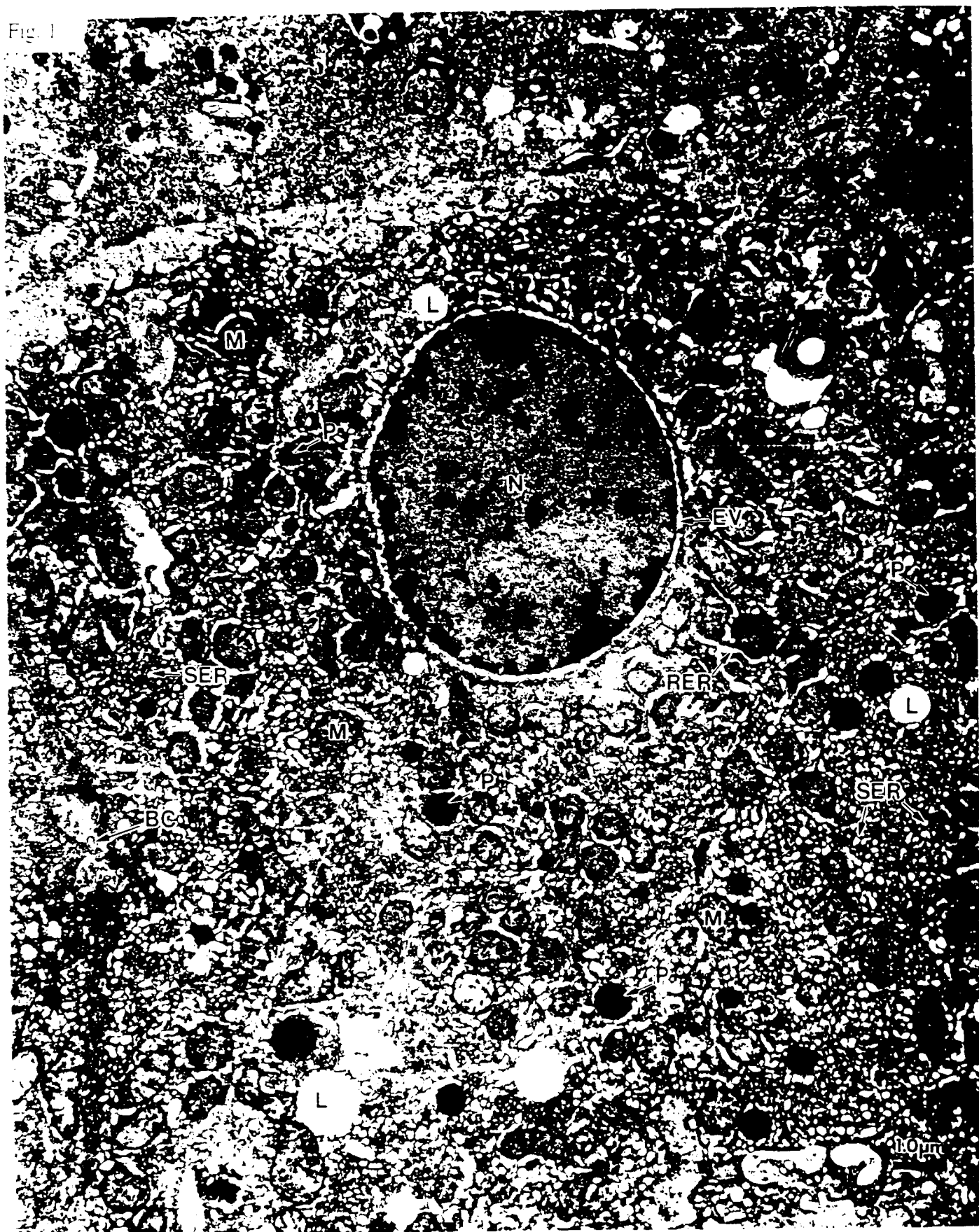
4 1/2

F -

21 <

FIGURE 1

Fig. 1



Study: 6329-223

PAI EM No.: 99.76-32-2A

Test Article No.: IO5511 U-Sac

Group/Sex: 4-M

Negative No.: Z18831

Magnification: 10,320x

Date: 5/22/00 Tech: NW

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Z18831

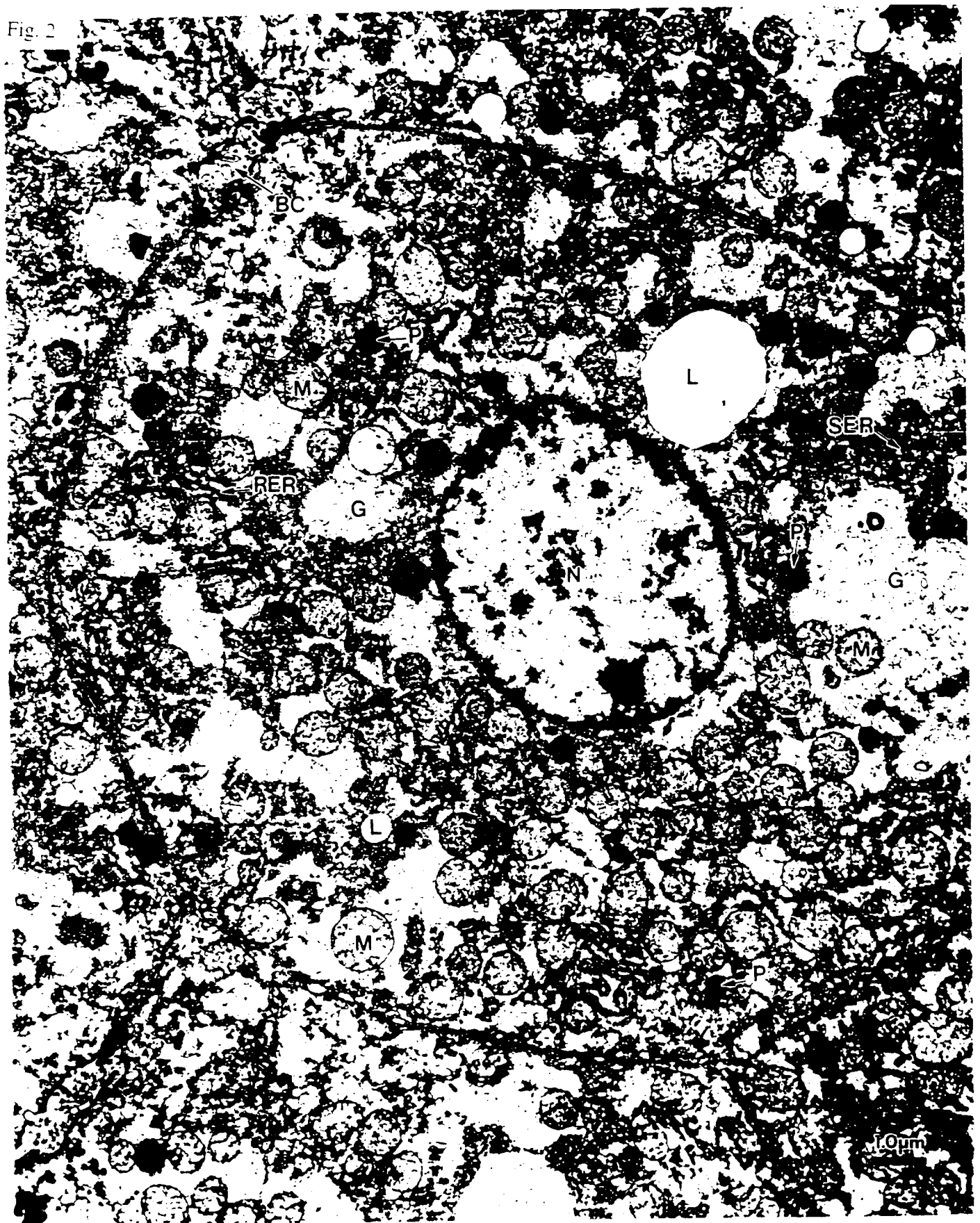
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45

FIGURE 2

Fig. 2



Study: 6329-223

PAI EM No.: 99.76-37-1A

Test Article No.: IO5542 Biopsy

Group/Sex: 4-F

Negative No.: Z18854

Magnification: 10,320x

Date: 5/22/00 Tech: NW

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Durham, NC 27713

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Z18854

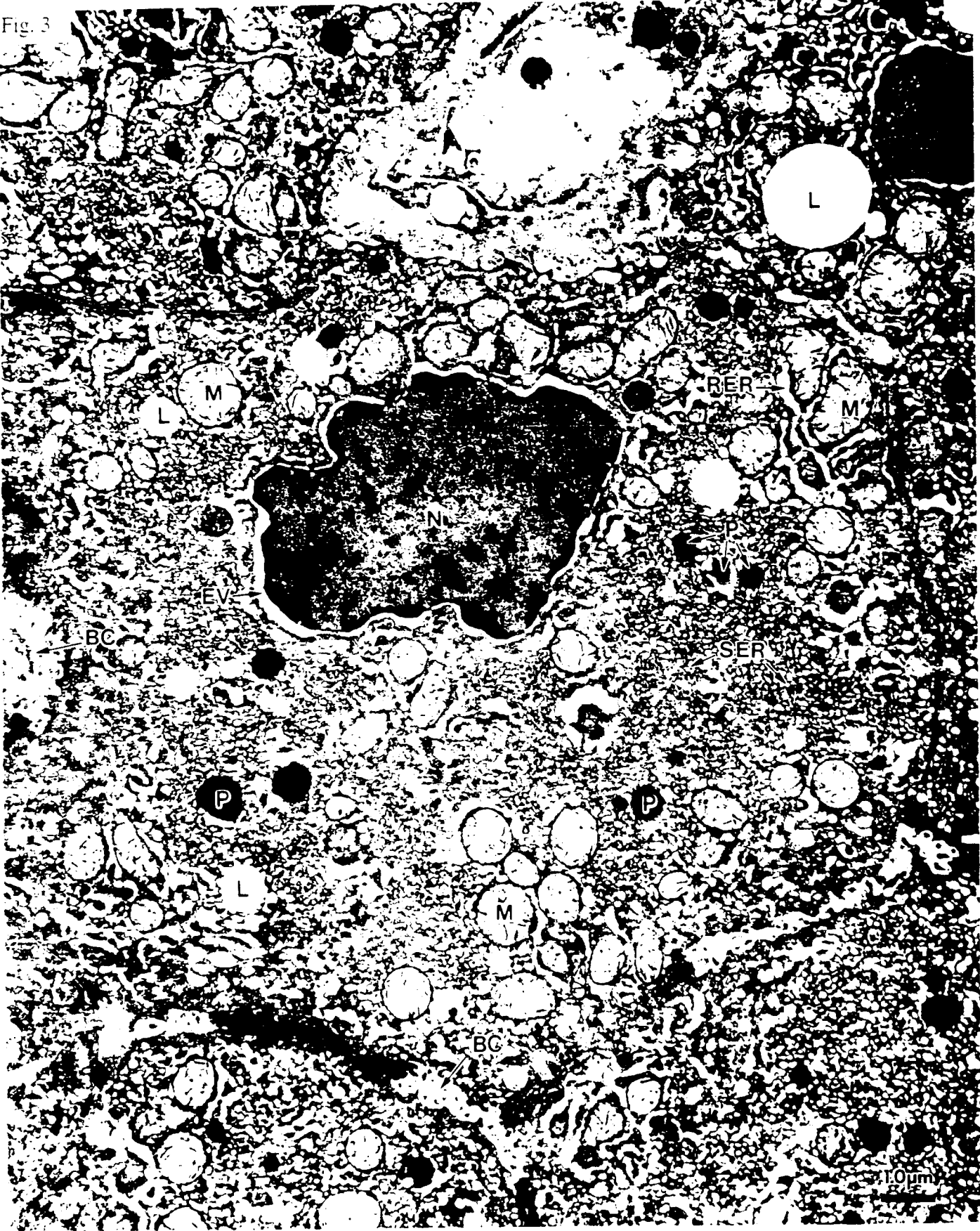
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F -

4 S

FIGURE 3

Fig. 3



Study: 6329-223

PAI EM No.: 99.76-36-2A

Test Article No.: IO5533 U-Sac

Group/Sex: 4-F

Negative No.: Z18849

Magnification: 10,320x

Date: 5/22/00 Tech: NW

Pathology Associates International

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Durham, NC 27713

(919) 544-5257

Z18849

4 1/2

F -

4_s

FIGURE 4

Fig. 4



Index of Labels for Ultrastructural Structures

<u>Abbreviation</u>	<u>Structure</u>
BC	Bile canaliculus
EV	Nuclear envelope
G	Glycogen
L	Lipid droplet
M	Mitochondria
N	Nucleus
P	Peroxisome
RB	Residual body
RER	Rough endoplasmic reticulum
SER	Smooth endoplasmic reticulum

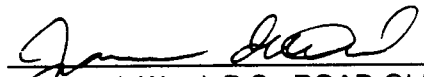
IV. Quality Assurance Statement



QUALITY ASSURANCE STATEMENT

This electron microscopy study has been inspected and audited by the Quality Assurance Unit as required by the Good Laboratory Practices Regulations promulgated by the U.S. Food and Drug Administration. PAI has a functioning and responsive Quality Assurance Unit which reports directly to management. The following is a record of inspections/audits and their resulting reports:

<u>Date of Inspection</u>	<u>Phase Inspected</u>	<u>Date Findings Reported to Management and Study Director</u>
4/1, 2/99	Data Audit	4/2/99
4/2/99	Draft Report Audit	4/2/99
7/13/99	Audit of Data from Additional Groups	7/13/99
7/13/99	Final Report Audit	7/13/99
5/25, 30/00	Data and Draft Report Audit Recovery Animals	5/30/00


Jeanne deWard, B.S., RQAP-GLP
Quality Assurance Specialist

5/30/00
Date

Sponsor: 3M Corporate Toxicology

Test Article: PFOS

Study Number: 6329-223, PAI Study EM-99.76