

INTERIM PATHOLOGY REPORT
24 months Post-Explant

WUXI APPTEC PROJECT NUMBER 112085

STUDY TITLE:
COMPARATIVE STUDY TO ASSESS A NOVEL TISSUE PRESERVATIVE SOLUTION

TEST ARTICLE IDENTIFICATION:
Novec (TM) 7100, Lot #: 20221

SPONSOR:
3M
Bld. 236-2B-01
St. Paul, MN 55144

TEST CODE:
C6726

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03/08/11

**COMPARATIVE STUDY TO ASSESS A NOVEL TISSUE PRESERVATIVE SOLUTION
24 months Post-Explant**

INTRODUCTION:

This report presents the results of histopathologic evaluation of hematoxylin and eosin (H&E) stained tissue sections and interpretation of anatomic histopathologic data from a **Comparative Study To Assess A Novel Tissue Preservative Solution**. The purpose of the study is to compare gross and microscopic tissue morphology following long term storage of a novel tissue preservation solution and to compare these findings to standard formalin fixed tissues.

OVERVIEW OF THE EXPERIMENTAL DESIGN AND METHODS: (Details provided in the Final Report).

Two rabbits, (which had already undergone previous testing in order to conserve on animals), were re-assigned to this project, testing a novel fixative. Both animals were perfused with formalin. At the end of the perfusion, selected tissues were collected at necropsy. Tissues from one animal were rinsed and stored in fresh 10% NBF (neutral buffered formalin). Tissues from the second animal were rinsed and stored in the test article (a novel tissue preservation solution).

Test Article Identification: Novec (TM) 7100, Lot #: 20221

Experimental Design:

Group	Animal ID	Animal Species (Strain)	Sex
10% NBF	8684	Rabbit (New Zealand White)	Male
Novec (TM) 7100, Lot #: 20221	8685	Rabbit (New Zealand White)	Male

Tissue Storage Time Points:

Time Post-Explant:
14 Days
90 Days
180 Days
12 Months
24 Months
36 Months
48 Months

HISTOPATHOLOGY:

At specified times post-fixing (see above), sections of selected tissues were processed using routine paraffin embedding methods. One section per tissue was stained with Hematoxylin and Eosin (H&E), and evaluated by a veterinary pathologist. A second section was collected unstained, without a cover slip, and given to the client for possible immunohistochemistry at a later date.

Tissues Submitted for Histology, and Slide Identification List:

Tissue	Slide
Heart	A
Liver	B
Eyes	C

A, B, and C refer to the slide label.

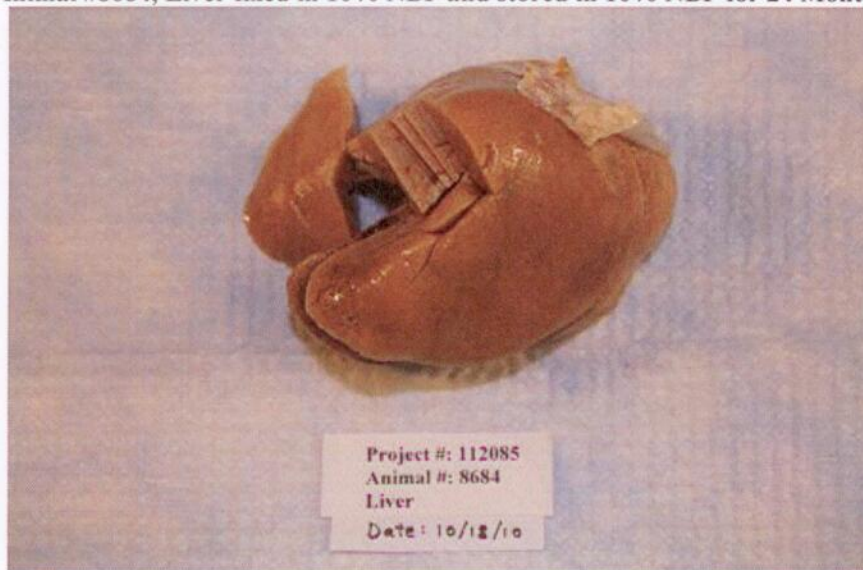
Histo ID: 10-3466

RESULTS AND DISCUSSION:

Comparison of tissues stored in 10% Neutral Buffered Formalin (NBF) (Animal #8684) to tissues stored in a novel tissue preservation solution (Novec (TM) 7100, Lot #: 20221) (Animal #8685):

Macroscopic findings: The color and consistency of the selected tissues (liver, heart, and eyes) were similar, when comparing tissues from Animal #8684 to tissues from Animal #8685 at 24 months post-explant. At 24 months, the heart from #8684 and #8685 was described as slightly pale but otherwise normal; the liver from #8684 and #8685 was described as pale, with brown mottling of the larger lobe; and the eyes from #8684 and #8685 were described as normal. In photographs, all three tissues at 24 months appeared paler compared to photographs of these tissues taken at 14 days post-explant, but were similar in color to photographs taken at 90 days, 180 days, and 12 months post-explant.

Animal #8684, Liver fixed in 10% NBF and stored in 10% NBF for 24 Months.



Animal #8685, Liver fixed in 10% NBF and stored in Novec (TM) 7100, Lot #: 20221 for 24 Months.



Animal #8684, Heart fixed in 10% NBF and stored in 10% NBF for 24 Months.



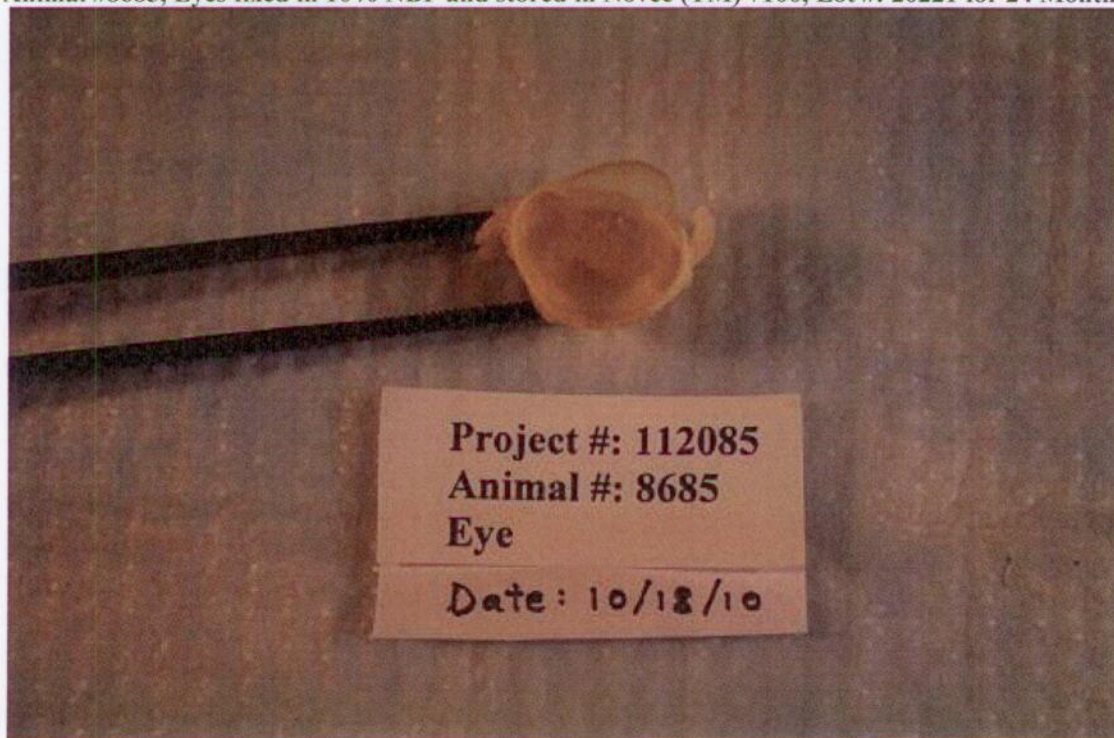
Animal #8685, Heart fixed in 10% NBF and stored in Novec (TM) 7100, Lot #: 20221 for 24 Months.



Animal #8684, Eyes fixed in 10% NBF and stored in 10% NBF for 24 Months.



Animal #8685, Eyes fixed in 10% NBF and stored in Novec (TM) 7100, Lot #: 20221 for 24 Months.



Microscopic Findings: Comparison of tissues stored in 10% Neutral Buffered Formalin (NBF) (Animal #8684) to tissues stored in a novel tissue preservation solution (Novec (TM) 7100, Lot #: 20221) (Animal #8685):

HEART:

There were no significant differences at 24 months post-explant in the staining characteristics of the heart in the histology sections when comparing #8684 to #8685. Compared to histology sections of the heart from earlier time-points, sections prepared at 24 months post-explant appeared similar to those prepared at 12 months post-explant. Compared to the earlier time points, (14 days, 90 days and 180 days), there appeared to be a loss in cellular detail in the heart sections from both animals. Note: For ease of comparison, photographs included below were from areas of the heart that were normal.

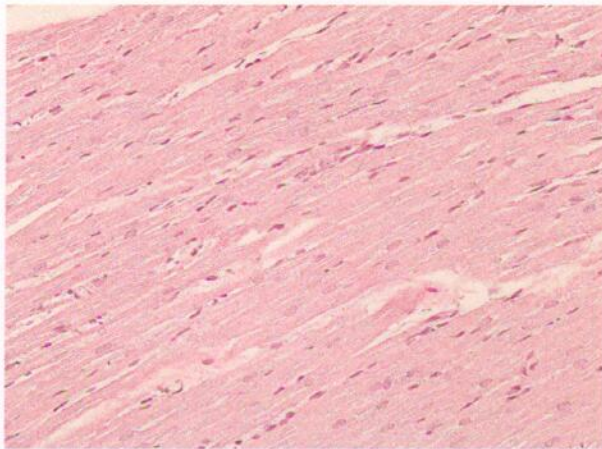


Fig. 1A: Animal #8684, Heart fixed in 10% NBF and stored in 10% NBF for 24 months; stained with H&E, 20x magnification.

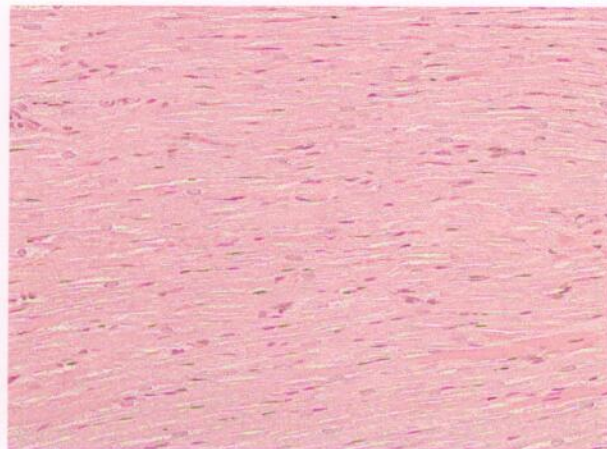


Fig. 1C: Animal #8685, Heart fixed in 10% NBF and stored in Novec (TM) 7100, Lot #: 20221 for 24 months; stained with H&E, 20x magnification.

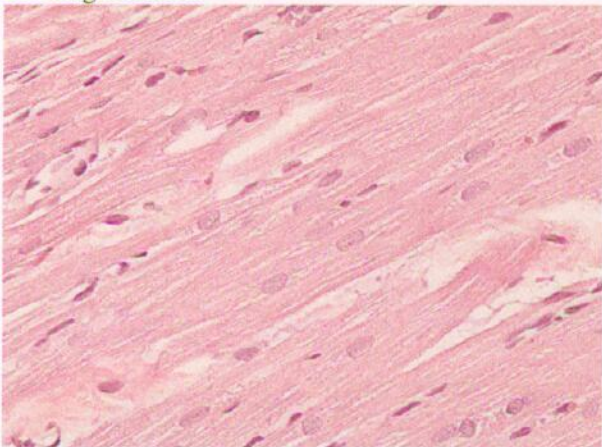


Fig. 1B: Animal #8684, Heart fixed in 10% NBF and stored in 10% NBF for 24 months; stained with H&E, 40x magnification.

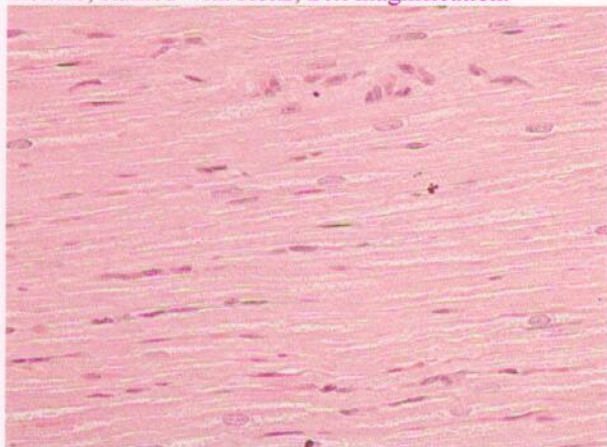


Fig. 1D: Animal #8685, Heart fixed in 10% NBF and stored in Novec (TM) 7100, Lot #: 20221 for 24 months; stained with H&E, 40x magnification.

LIVER:

Differences in the staining characteristics of liver sections at 24 months post-explant appeared minimal when comparing #8684 to #8685. Compared to earlier time-points, histology sections of the liver from #8684 and #8685 appeared similar to those prepared at the 12 month time-point, but appeared paler than sections prepared at earlier time-points (14 days, 90 days, and 180 days), and had less cellular detail (cell outlines and nuclear outlines were less crisp, although nucleoli were still discernable within the cell nuclei). Note: For ease of comparison, photographs included below were from areas of the liver that were relatively normal.

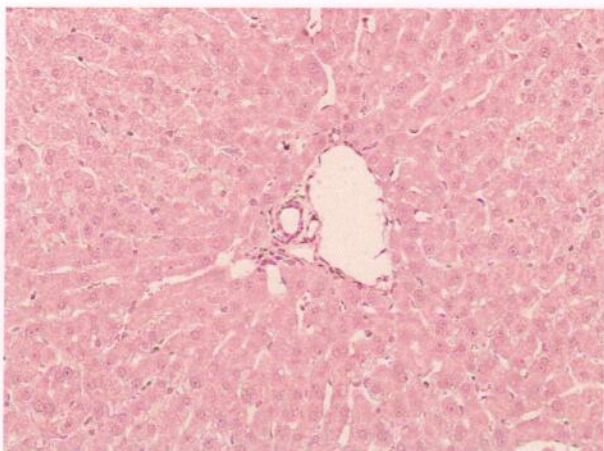


Fig. 2A: Animal #8684, Liver fixed in 10% NBF and stored in 10% NBF for 24 months; stained with H&E, 20x magnification.

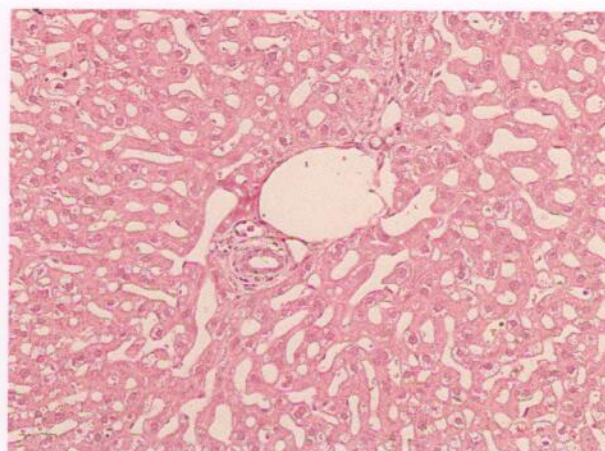


Fig. 2C: Animal #8685, Liver fixed in 10% NBF and stored in Novec (TM) 7100, Lot #: 20221 for 24 months; stained with H&E, 20x magnification.

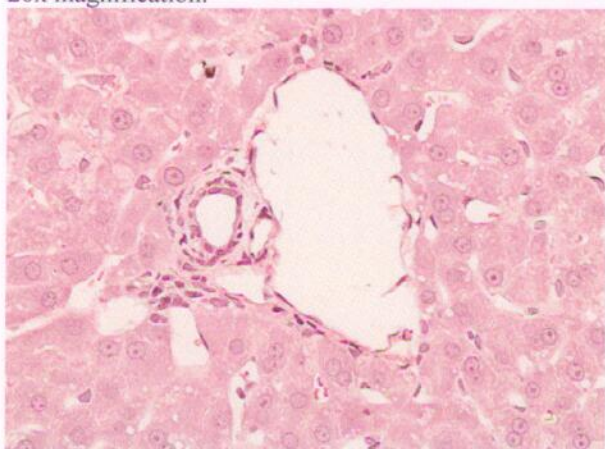


Fig. 2B: Animal #8684, Liver fixed in 10% NBF and stored in 10% NBF for 24 months; stained with H&E, 40x magnification.

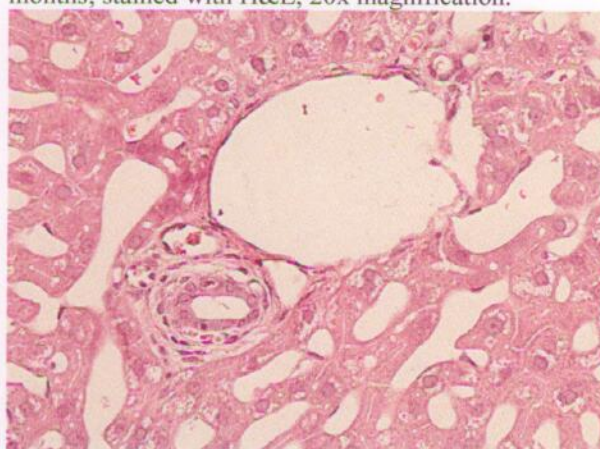


Fig. 2D: Animal #8685, Liver fixed in 10% NBF and stored in Novec (TM) 7100, Lot #: 20221 for 24 months; stained with H&E, 40x magnification.

EYES:

There were no significant differences in the staining characteristics of the eyes in the histology sections when comparing #8684 to #8685 at 24 months to each other. Sections were similar to those prepared at 12 months post-explant, and were paler and less crisp than tissue sections prepared from earliest time-points (14 days, 90 days, 180 days). Note: Sections from both animals were fragmented and the retinas were detached (processing artifacts).



Fig. 3A: Animal #8684, Eye fixed in 10% NBF and stored in 10% NBF for 24 months; stained with H&E, 10x magnification.

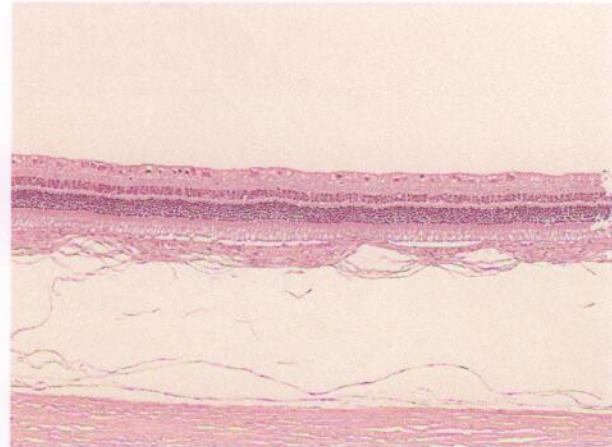


Fig. 3C: Animal #8685, Eye fixed in 10% NBF and stored in Novec (TM) 7100, Lot #: 20221 for 24 months; stained with H&E, 10x magnification.

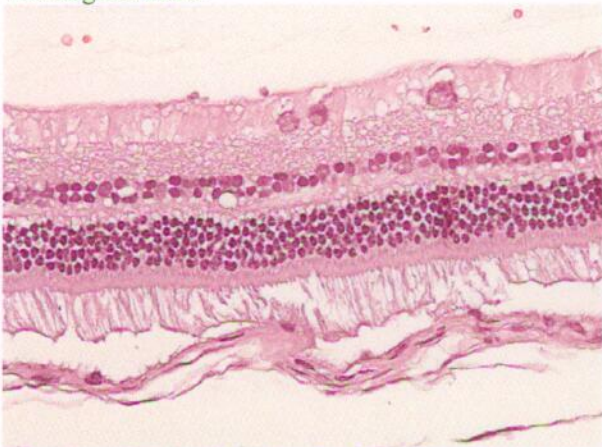


Fig. 3B: Animal #8684, Eye fixed in 10% NBF and stored in 10% NBF for 24 months; stained with H&E, 40x magnification.

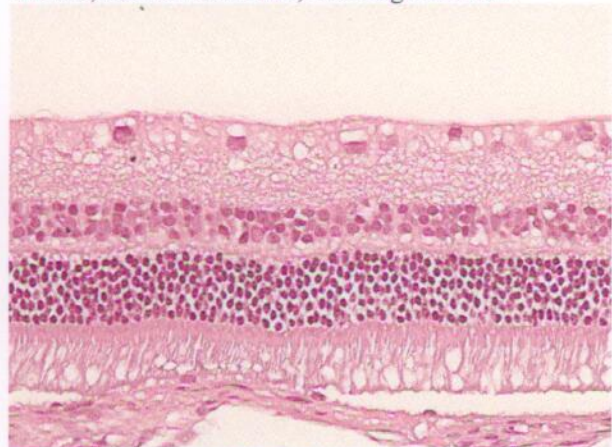


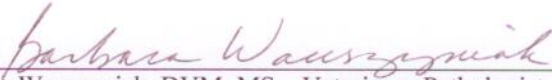
Fig. 3D: Animal #8685, Eye fixed in 10% NBF and stored in Novec (TM) 7100, Lot #: 20221 for 24 months; stained with H&E, 40x magnification.

SUMMARY AND CONCLUSION:

Under the conditions of the study, there were no significant macroscopic differences in tissues, (liver, heart, and eye), stored in **Novec (TM) 7100, Lot #: 20221**, (#8685), compared to tissues, (liver, heart, and eye), stored in formalin, (#8684), for 24 months.

There were no significant differences in staining characteristics in histology sections from the liver, heart and eye between #8684 (stored in formalin) and #8685 (stored in **Novec (TM) 7100, Lot #: 20221**). Histology sections from the eye from both animals were fragmented, which was attributed to processing artifacts, rather than to differences in fixation.

At 24 months post-fixation, histology sections of the liver, heart and eyes from both #8684 and #8685 had decreased staining intensity, (stained paler), and the reduced cellular detail, (cells and nuclei were less crisp in outline, although nucleoli were still visible in liver sections), than at the earliest time-points (14 days, 90 days and 180 days).



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03/08/11

Date