

VIII.c.3. Non-Serious Day 1-7 Probably Device Related AEs (window 1-21 days) – By Event Category



VIII.c.4. Non-Serious 6 Week Probably Device Related AEs (window 22-63 days) - By  
Event Category



VIII.c.5. Non-Serious 3 Month Probably Device Related AEs (window 64-136 days) - By Event Category



VIII.c.6. Non-Serious 6 Month Probably Device Related AEs (window 137-273 days) -  
By Event Category



VIII.c.7. Non-Serious 12 Month Probably Device Related AEs (window 274-547 days) -  
By Event Category



VIII.d.1. Non-Serious Possibly Device Related AEs  
By Time Period



VIII.d.2. Non-Serious Day 1-7 Possibly Device Related AEs (window 1-21 days) - By



VIII.d.3. Non-Serious 6 Week Possibly Device Related AEs (window 22-63 days) - By  
Event Category



VIII.d.4. Non-Serious 3 Month Possibly Device Related AEs (window 64-136 days) - By  
Event Category



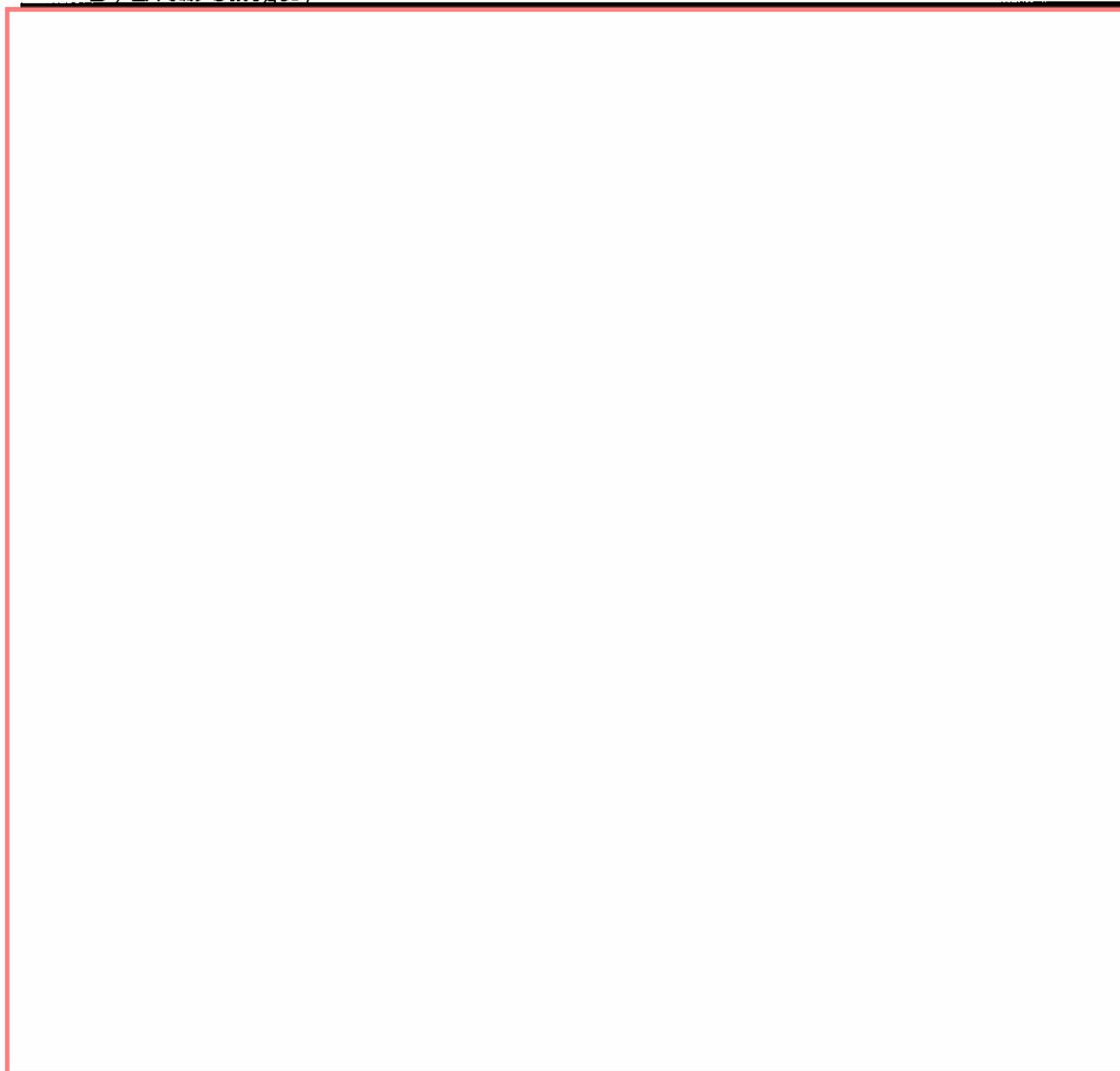
VIII.d.5. Non-Serious 6 Month Possibly Device Related AEs (window 137-273 days) -  
By Event Category



VIII.d.6. Non-Serious 12 Month Possibly Device Related AEs (window 274-547 days) -  
By Event Category



VIII.d.7. Non-Serious 24 Month Possibly Device Related AEs (window 548-912 days) -  
By Event Category



VIII.d.8. Non-Serious >24 Month Possibly Device Related AEs (window >912 days) -  
By Event Category



VIII.e.1. Non-Serious Unknown Device Relatedness AEs  
By Time Period



**VIII.e.2. Non-Serious Operative Unknown Device Relatedness AEs (day 0) - By Event Category**



**VIII.e.3. Non-Serious Day 1-7 Unknown Device Relatedness AEs (window 1-21 days) -  
By Event Category**



VIII.e.4. Non-Serious 6 Week Unknown Device Relatedness AEs (window 22-63 days) -  
By Event Category



VIII.e.5. Non-Serious 3 Month Unknown Device Relatedness AEs (window 64-136 days)  
By Event Category



VIII.e.6. Non-Serious 6 Month Unknown Device Relatedness AEs (window 137-273 days) - By Event Category



VIII.e.7. Non-Serious 12 Month Unknown Device Relatedness AEs (window 274-547 days) - By Event Category



VIII.e.8. Non-Serious 24 Month Unknown Device Relatedness AEs (window 548-912 days) - By Event Category



VIII.e.9. Non-Serious >24 Month Unknown Device Relatedness AEs (window >912 days) - By Event Category



VIII.f.1. Non-Serious Not Related to The Device AEs  
By Time Period



**VIII.f.2. Non-Serious Operative Not Related to The Device AEs (day 0) – By Event Category**

A large rectangular area that has been redacted, indicated by a red border. It is intended to contain data for the section VIII.f.2.

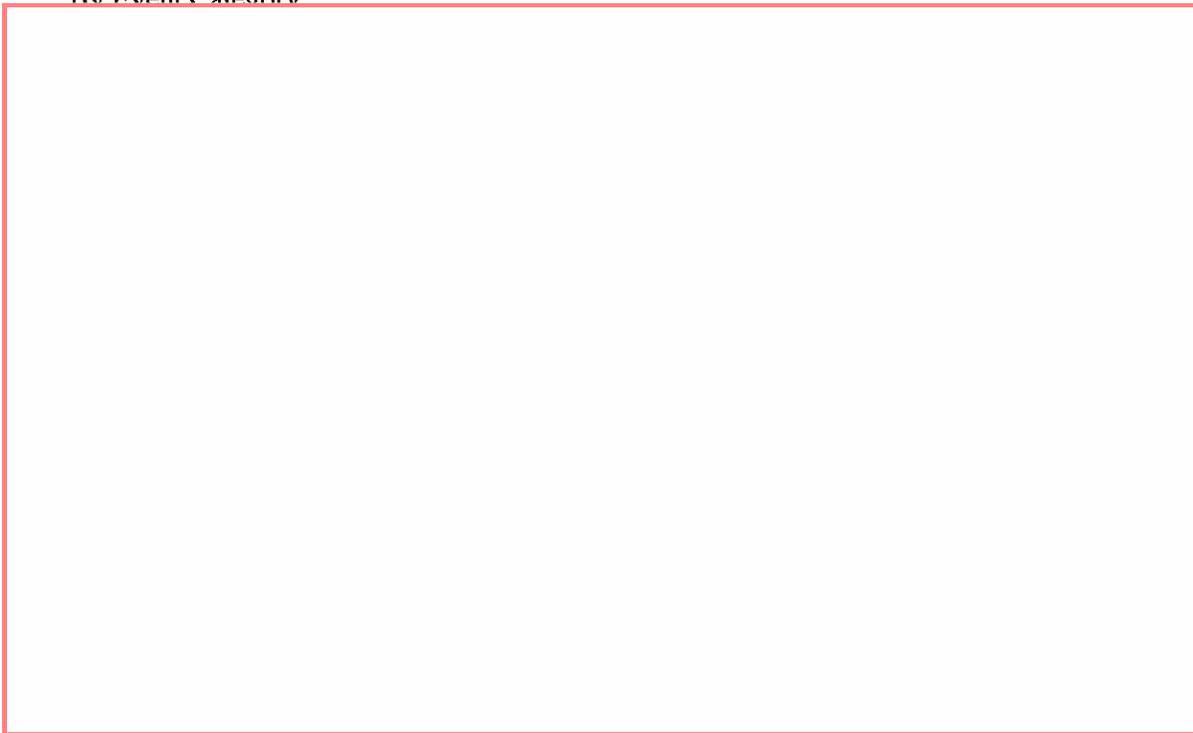
**VIII.f.3. Non-Serious Day 1-7 Not Related to The Device AEs (window 1-21 days) – By Event Category**

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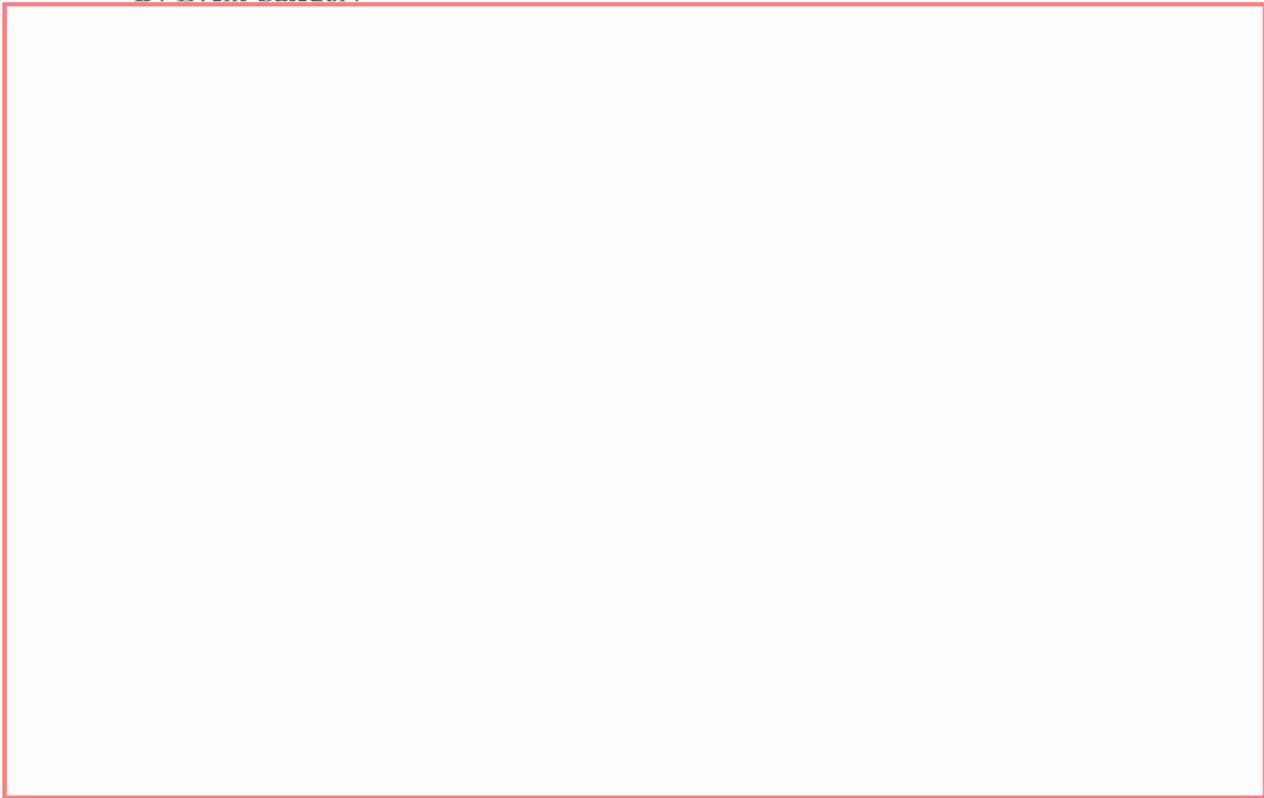
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VIII.f.5. Non-Serious 3 Month Not Related to The Device AEs (window 64-136 days) –  
By Event Category



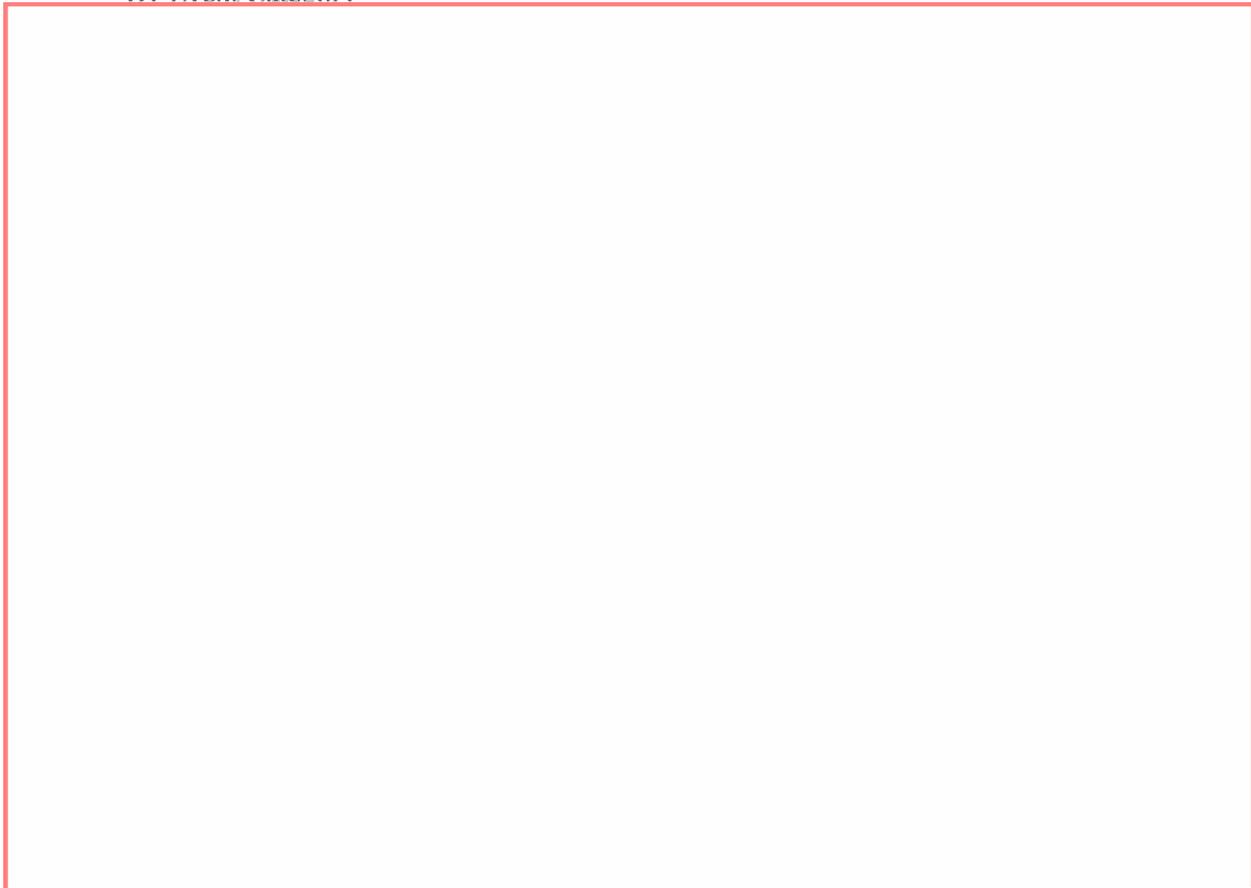
VIII.f.6. Non-Serious 6 Month Not Related to The Device AEs (window 137-273 days) –  
By Event Category



VIII.f.7. Non-Serious 12 Month Not Related to The Device AEs (window 274-547 days)



VIII.f.8. Non-Serious 24 Month Not Related to The Device AEs (window 548-912 days)  
– By Event Category



VIII.f.9. Non-Serious >24 Month Not Related to The Device AEs (window >912 days) --  
By Event Category



## Appendix D

### Reoperations in Control Patients







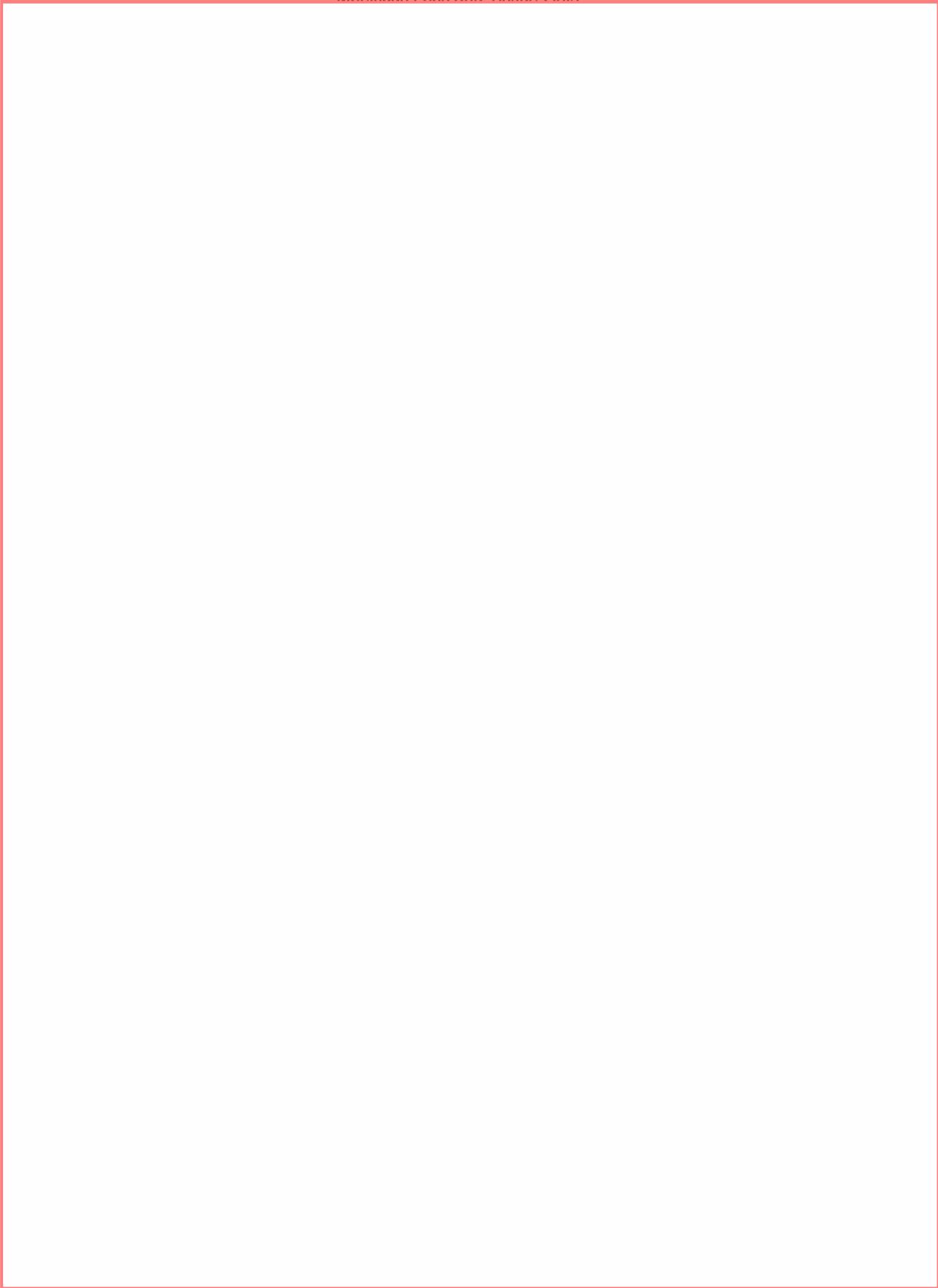
## Appendix E

### Line Listing of Tissue Loss and Tissue Gain



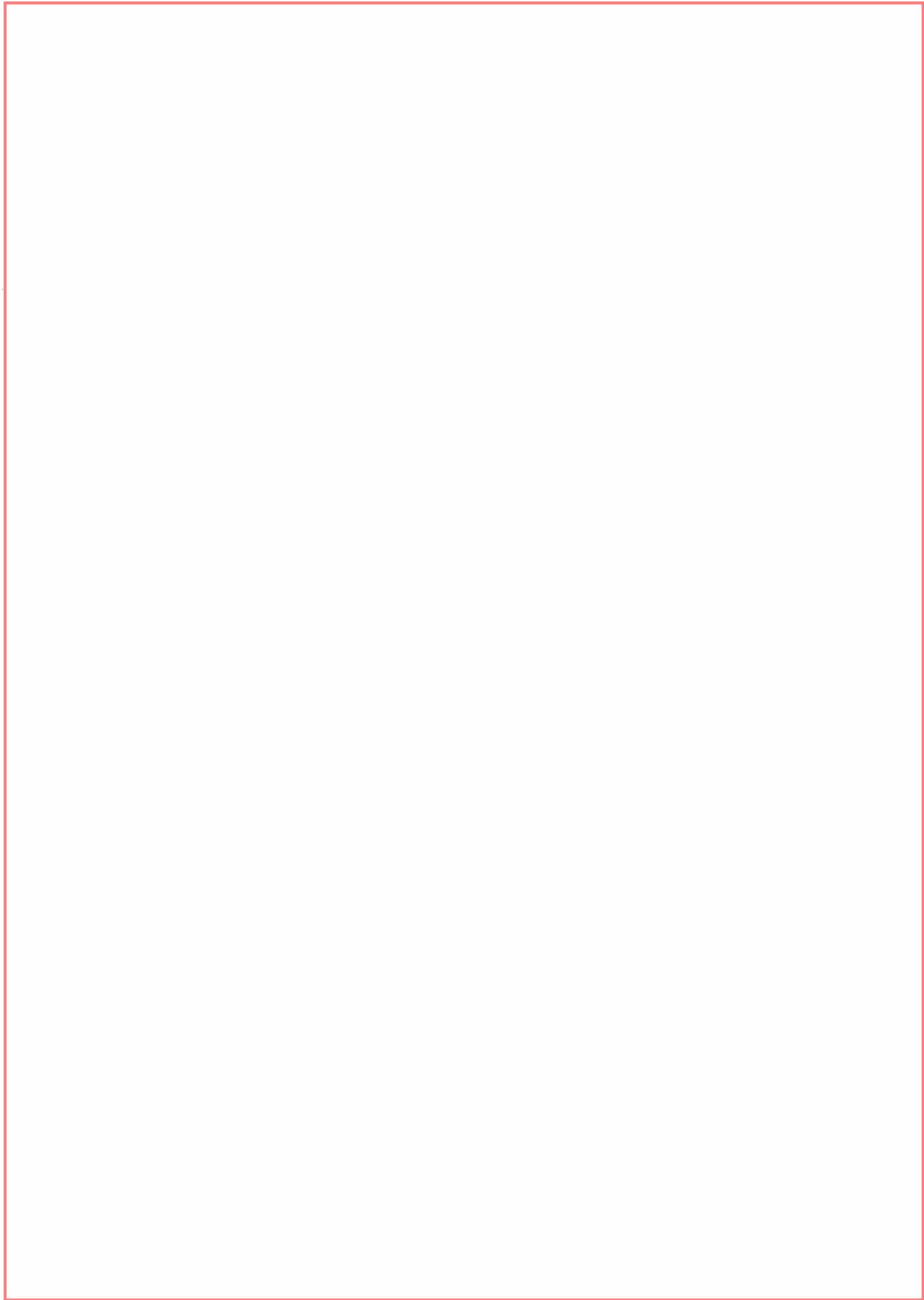
ReGen Biologics, Inc. (CMI Study data as   
Meniscus Loss and Tissue Gain  
By Patient







ReGen Biologics, Inc. (CMI Study data as of )  
Meniscus Loss and Tissue Gain



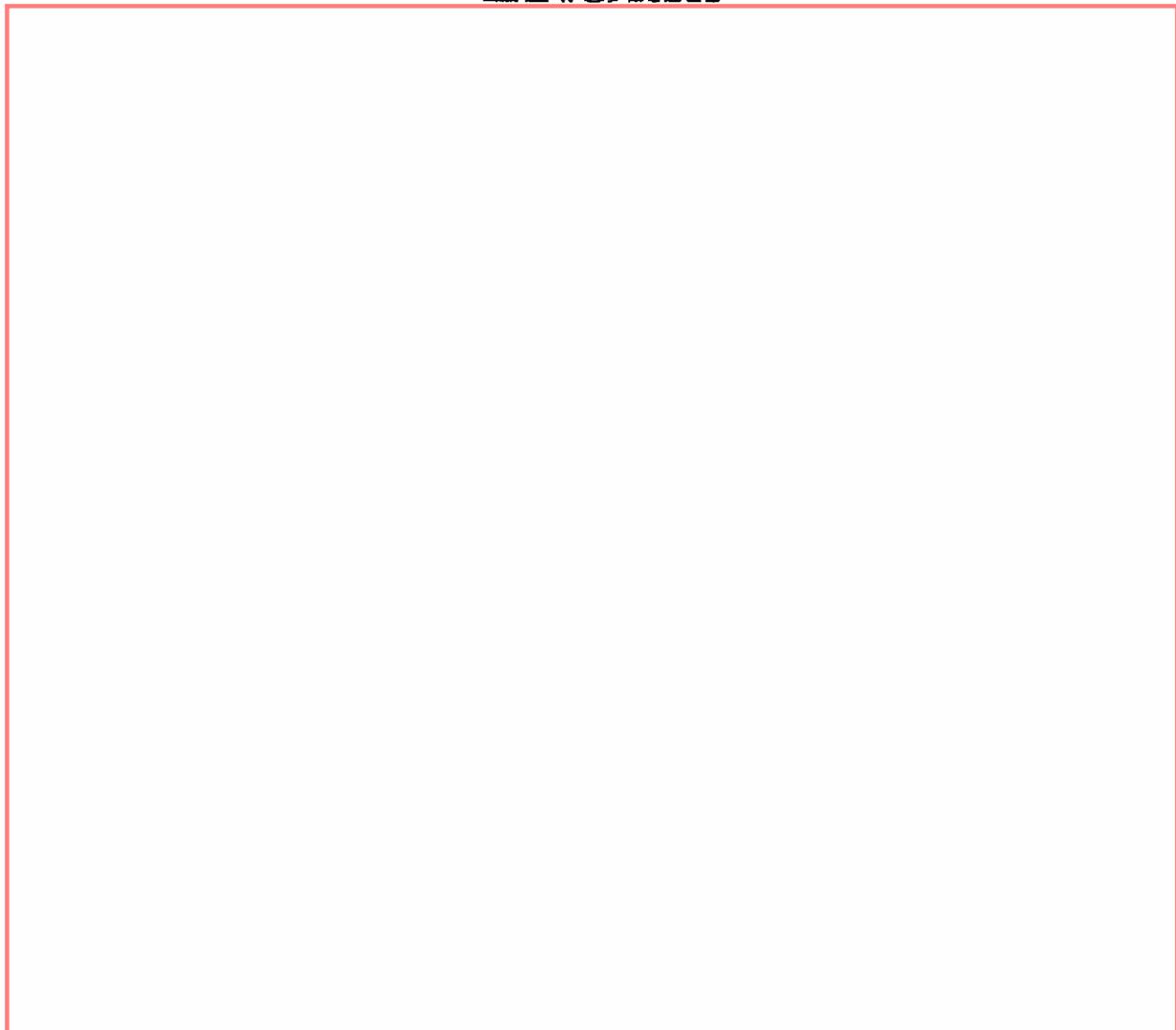
ReGen Biologics, Inc. (CMI Study data as of )  
Meniscus Loss and Tissue Gain  
By Patient



## Appendix F

### Report of Histological Evaluation

**Histologic Evaluation of Biopsy Samples from Patients  
Enrolled in the U.S. Multicenter Clinical Trial  
Of the Collagen Meniscus Implant  
IDE #G920211**



## Introduction

Meniscal injuries are common and if not corrected a potential source of osteoarthritis of the knee. The loss of the protective effects of the meniscus result in articular cartilage damage and impaired knee function. The collagen meniscus implant (CMI) is a porous type I bovine collagen scaffold developed by ReGen Biologics which is surgically sutured to the medial meniscus rim. The CMI provides support to the meniscus after removal of damaged tissue and a scaffold for replacement by the patient's own tissue.

A randomized, controlled, multicenter clinical trial [redacted] conducted to evaluate the use of the CMI in patients age 18 to 60 years of both sexes with meniscus deficiencies resulting from irreparable tears of the medial meniscus. The study was divided into two arms, one arm was for patients who had no previous treatment to the involved meniscus and the other was for patients with from one to three previous treatments to the involved meniscus. For purposes of this histologic analysis, all patients who received the CMI are evaluated as a single treatment group.

## Materials and Methods

A total of 313 patients were enrolled and treated under the clinical protocol. Of these patients, 162 patients received the CMI and 151 patients received the control procedure, a partial meniscectomy. The protocol required all patients who received the CMI to return one year post surgery for a relook arthroscopy and biopsy to assess the condition of the implant and the tissue that replaced it. 135 (83.3%) of the 162 CMI patients had biopsy samples taken at the time of relook surgery or at the time of explantation. Needle biopsies directed at the interface region of the CMI and native meniscus were performed at the time of relook arthroscopy, under direct visual observation, using [redacted]. These yielded a specimen for examination of approximately 1.5mm in diameter and varying lengths. [redacted]

[redacted]  
report the histologic findings from these 136 biopsies in 135 patients (1 patient had two biopsies).

## Results

Of the 136 biopsies [redacted]  
[redacted]

[Redacted]

with none

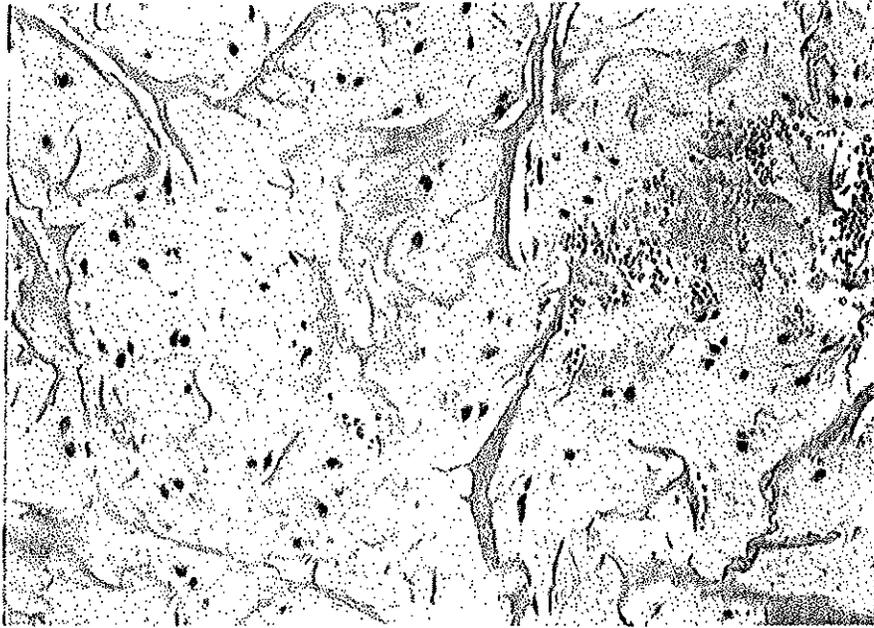
showing an adverse reaction except one non-specific synovitis.



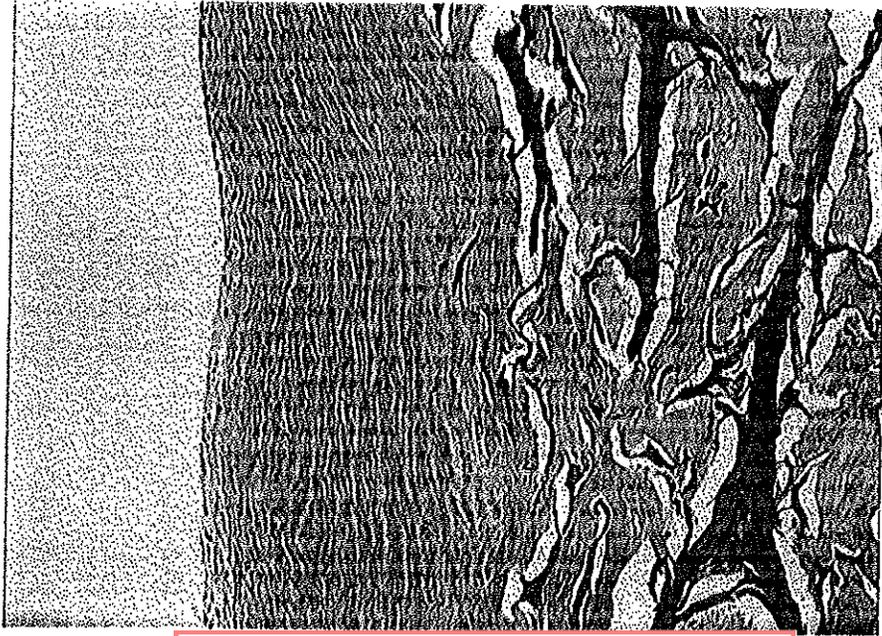
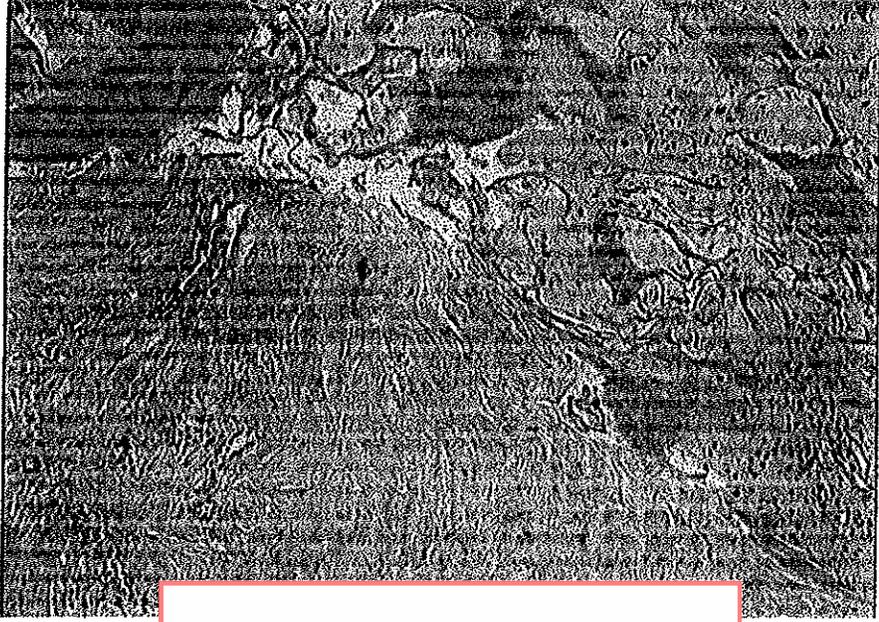
### **Conclusion**

In summary, the CMI appears to provide a scaffold for a predictable benign process of meniscal-like fibrochondrocytic matrix production by the host, and the CMI is integrated into this tissue as it is assimilated and resorbed. Healing incorporation into host tissues is demonstrable in this study. Except for a rarely observed inflammatory synovitis and implant inflammation, CMIs were not associated with a significant adverse reaction out to 12 months post placement of the device.

Figures



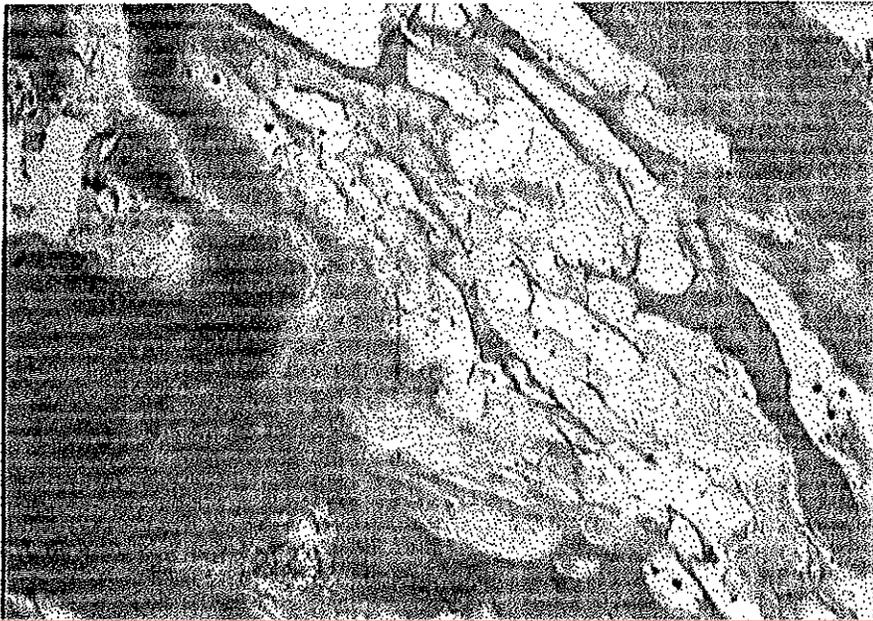
Figures



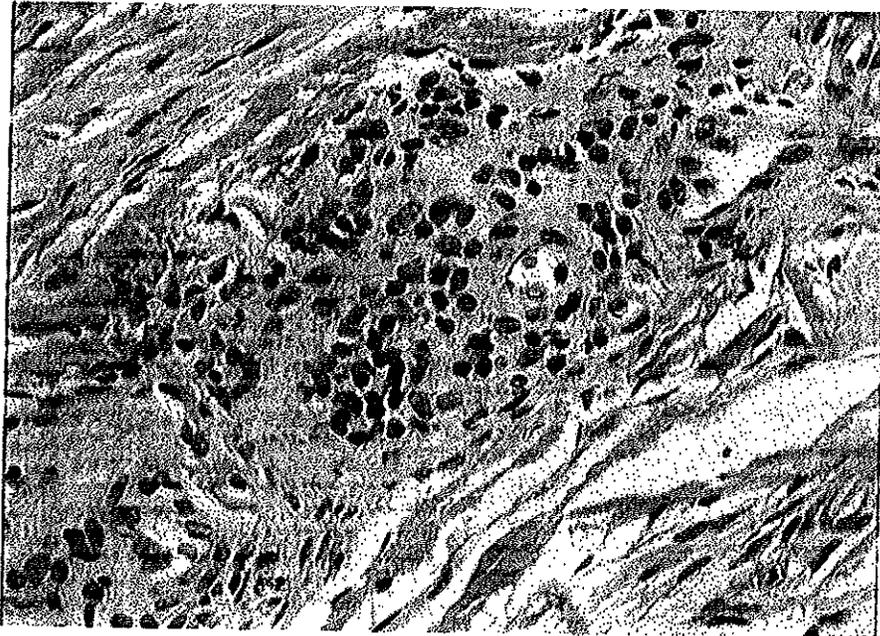
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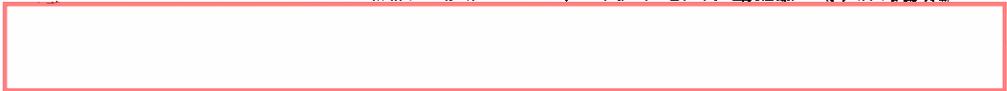
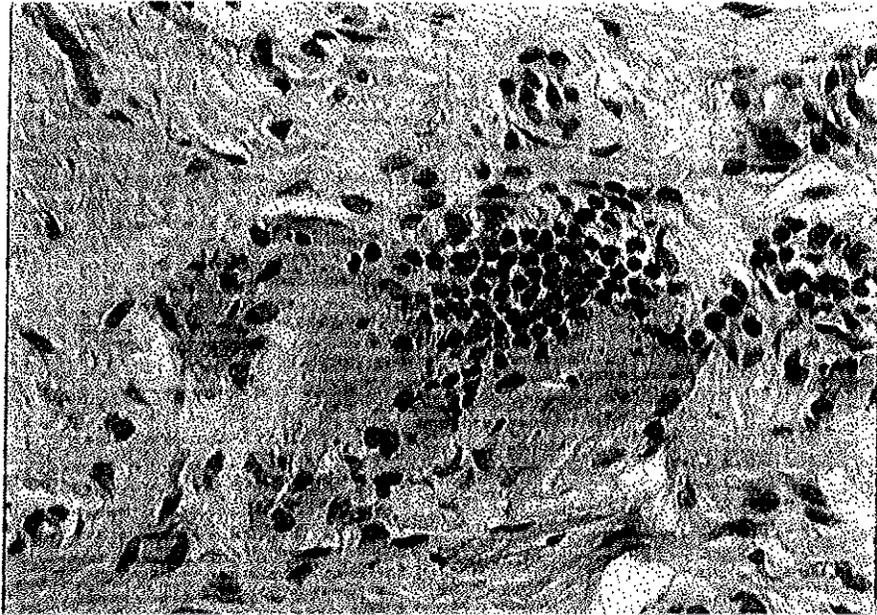
Figures



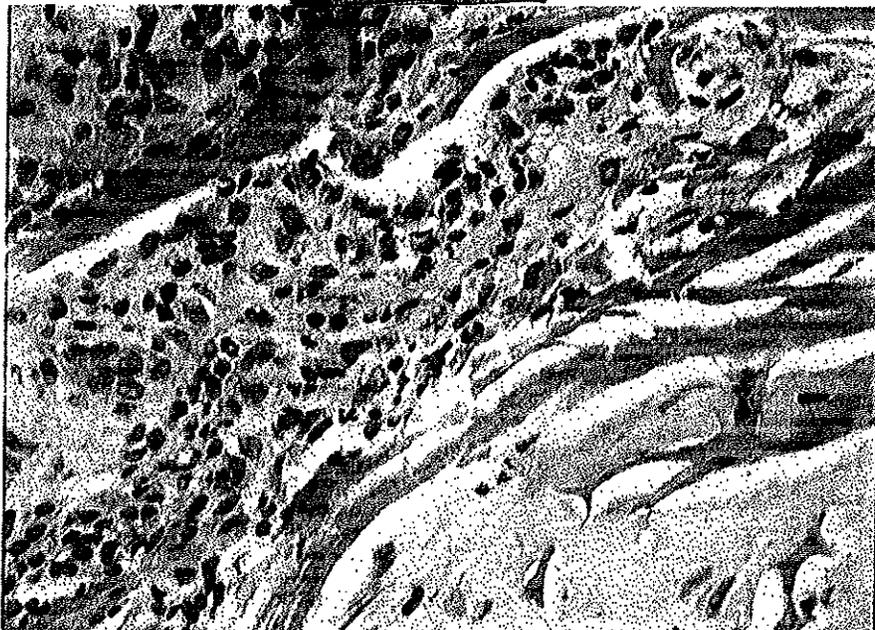
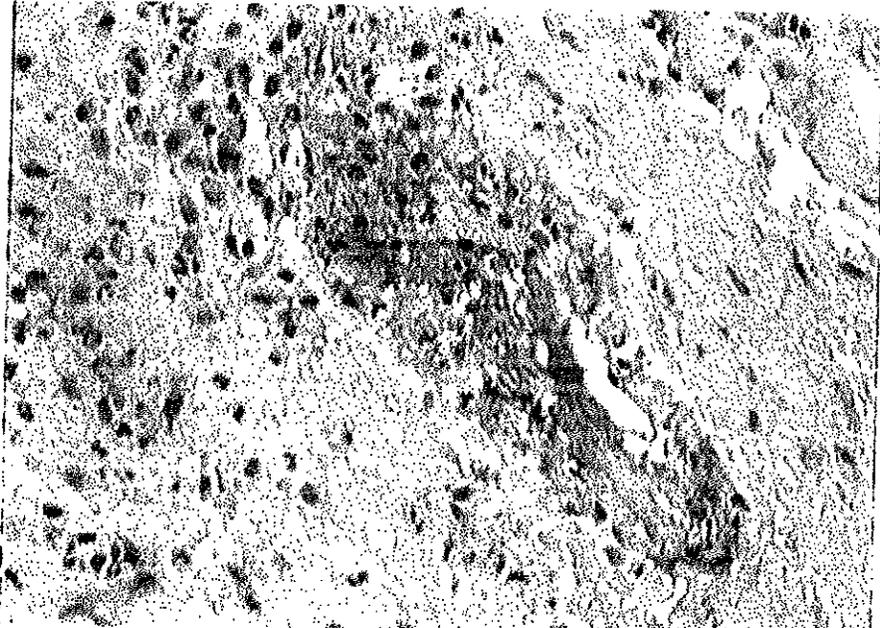
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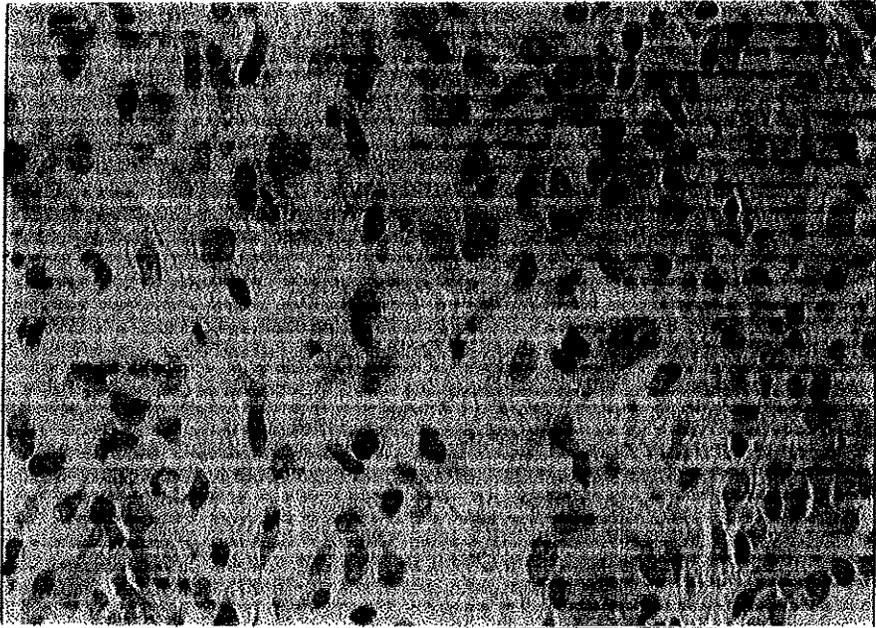
Figures



Figures



Figures



## Appendix G

### Narratives for Patients who Have Undergone Explants

**Explants reported in Protocols and 9601 and 9602**





## Appendix H

### Line Listing of All Adverse Events

Patient ID	Adverse Event Reported	Adverse Event Category	Serious	Relationship to Device
[Redacted Content]				

ReGen Biologics, Inc. (CMI Study data as of [REDACTED])  
listing of adverse events

Patient ID	Adverse Event Reported	Adverse Event Category	Serious	Relationship to Device
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Patient ID	Adverse Event Reported	Adverse Event Category	Serious	Relationship to Device
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Patient ID	Adverse Event Reported	Adverse Event Category	Serious	Relationship to Device

ReGen Biologics, Inc. (CMI Study data as of [redacted])  
listing of adverse events

Patient ID	Adverse Event Reported	Adverse Event Category	Serious	Relationship to Device
[Redacted Content]				

Patient ID	Adverse Event Reported	Adverse Event Category	Serious	Relationship to Device
[Redacted Content]				

ReGen Biologics, Inc. (CMI Study data as   
listing of adverse events

Patient ID	Adverse Event Reported	Adverse Event Category	Serious	Relationship to Device

Patient ID	Adverse Event Reported	Adverse Event Category	Serious	Relationship to Device
[Redacted Content]				

ReGen Biologics, Inc. (CMI Study data as   
listing of adverse events

Patient ID	Adverse Event Reported	Adverse Event Category	Serious	Relationship to Device

ReGen Biologics, Inc. (CMI Study data as of [redacted])  
listing of adverse events

Patient ID	Adverse Event Reported	Adverse Event Category	Serious	Relationship to Device
[Redacted Content]				

ReGen Biologics, Inc. (CMI Study data as listing of adverse events )

Patient ID	Adverse Event Reported	Adverse Event Category	Serious	Relationship to Device

Patient ID	Adverse Event Reported	Adverse Event Category	Serious	Relationship to Device

Patient ID	Adverse Event Reported	Adverse Event Category	Serious	Relationship to Device

ReGen Biologics, Inc. (CMI Study data as   
listing of adverse events

Patient ID	Adverse Event Reported	Adverse Event Category	Serious	Relationship to Device

Patient ID	Adverse Event Reported	Adverse Event Category	Serious	Relationship to Device

Patient ID	Adverse Event Reported	Adverse Event Category	Serious	Relationship to Device

Patient ID	Adverse Event Reported	Adverse Event Category	Serious	Relationship to Device
[Redacted Content]				

ReGen Biologics, Inc. (CMI Study data as )  
listing of adverse events

Patient ID	Adverse Event Reported	Adverse Event Category	Serious	Relationship to Device

ReGen Biologics, Inc. (CMI Study data as   
listing of adverse events

Patient ID	Adverse Event Reported	Adverse Event Category	Serious	Relationship to Device

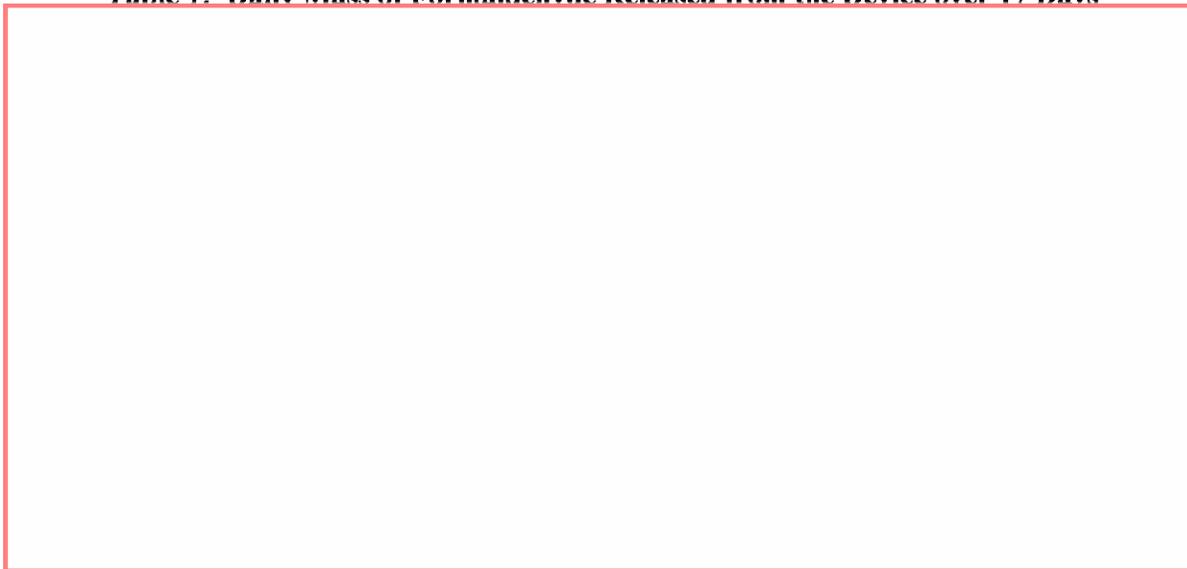
## Appendix I

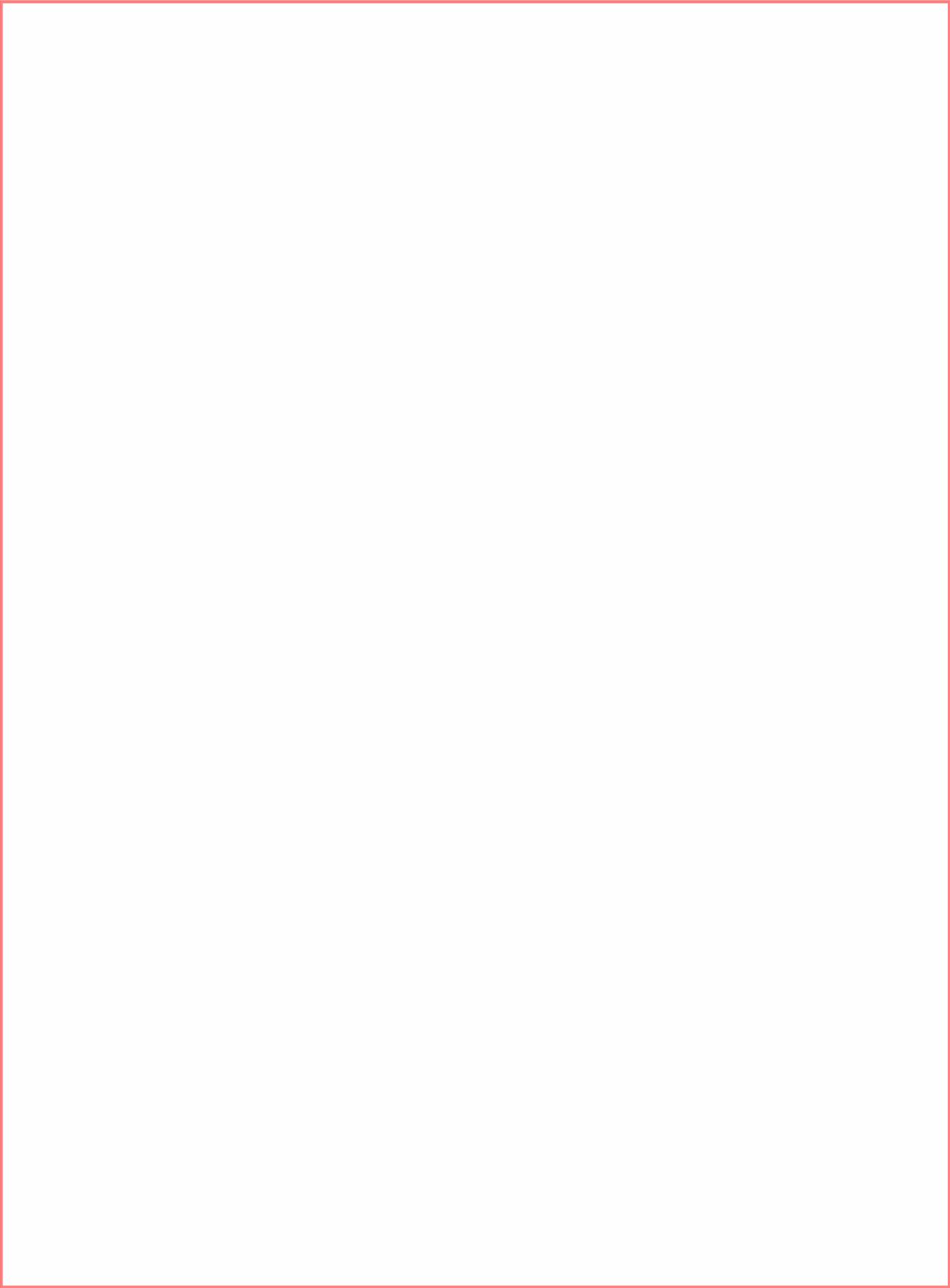
### Formaldehyde Residual Testing and Genotoxicity Testing

**RELEASE OF FORMALDEHYDE FROM CS AND  
ESTIMATED EXPOSURE TO PATIENT**



**Table 1: Daily Mass of Formaldehyde Released from the Device over 17 Days**







**Conclusion**









## References

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2. Simkin PA, Pizzorno JE. Transsynovial exchange of small molecules in normal human subjects. *J Appl Physiol* 36:581-587, 1974.
3. FDA Letter to ReGen Dated October, 14, 2004.
4. DHHS/ATSDR; Toxicological Profile for Formaldehyde (1999)
5. Hazardous Substances Data Bank, National Library of Medicine.
6. Sullivan, J.B. Jr., G.R. Krieger (eds.). *Hazardous Materials Toxicology-Clinical Principles of Environmental Health*. Baltimore, MD: Williams and Wilkins, 1992.
7. FDA Guidance Document - Q3C Impurities: Residual Solvents.
8. U.S. Environmental Protection Agency's Integrated Risk Information System (IRIS) on Formaldehyde (50-00-0).
9. Wangenheim J, and Bolcsfoldi, G. Mouse Lymphoma L5178Y Thymidine Kinase Locus Assay of 50 Compounds. *Mutagenesis* 3(3):193-205, 1988.
10. Grantly DC, et. al.; Mode of Mutagenic Action for the Biocide Bioban CS-1246 in Mouse Lymphoma Cells and Implications for Its In Vivo Mutagenic Potential. *Toxicological Sciences* 84(1): 73-80, 2005.
11. Specific Aspects of Regulatory Genotoxicity Tests, ICH Harmonized Tripartite Guideline S2A, Step 5, 19 July 1995 (Not supplied). Published in the Federal Register, Vol. 61, April 24, 1996, page 18199
12. Introduction to the OECD Guidelines on Genetic Toxicology Testing, 1997, section 5.4 (Not supplied)
13. Evaluation of Potential Carcinogenic Risks Posed by Formaldehyde Present in the Collagen Meniscus Implant, prepared by Brian Rogers, Ph.D., Diplomate, American Board of Toxicology.

**ATTACHMENT 1**  
**MATHEMATICAL MODEL**

**Mathematical Model Describing Equilibrium Concentration of Formaldehyde in the  
Knee Based on the In Vitro Release Model**



## Appendix J

### USP Heavy Metals Results

**Chromatographic System** (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 2-mm × 2-m column packed with 3% liquid phase G3 on silanized packing S1A and is maintained at 120°. Nitrogen is used as the carrier gas, flowing at the rate of about 30 mL per minute.

**Procedure**—Inject equal volumes (within the range of 2 µL to 20 µL) of the *Standard Preparation* and the *Test Preparation* into the chromatograph; record the chromatograms, and measure the areas for the major peaks. The ratio of the response of any dimethylaniline peak to the response of the naphthalene peak obtained from the *Test Preparation* is not greater than that obtained from the *Standard Preparation* (0.002%).

### (226) 4-EPIANHYDRO-TETRACYCLINE

This chromatographic procedure is provided to demonstrate that the content of 4-epianhydrotetracycline, a degradation product of tetracycline, does not exceed the limit given in the individual monograph.

**EDTA Buffer**—Dissolve 37.2 g of edetate disodium in 800 mL of water, adjust with ammonium hydroxide to a pH of 7.8, dilute with water to 1000 mL, and mix.

**Support Phase**—Add 5 mL of *EDTA Buffer* to 10 g of acid-washed chromatographic siliceous earth for column chromatography, and mix until the siliceous earth is uniformly moistened.

**Test Solution**—Prepare as directed in the individual monograph.

**Procedure**—Prepare a 15-mm × 170-mm chromatographic tube with a 4-mm × 50-mm outlet by packing it, in increments, with *Support Phase*, firmly tamping down each increment, until the tube is filled to a height of about 10 cm. In a beaker, prepare a mixture of 1 g of acid-washed chromatographic siliceous earth for column chromatography and 1 mL of *Test Solution*. Transfer the mixture to the top of the column. Dry-wash the beaker with *Support Phase*, and transfer to the column to provide an additional 1-cm layer on top of the mixture containing the *Test Solution*. Within 30 minutes, pass chloroform through the column, and collect successive fractions of 5.0 mL, 5.0 mL, 10.0 mL, 10.0 mL, and 5.0 mL. Observe the column during elution, and note the appearance of two separate yellow bands. The fraction or fractions containing the first yellow band contain the anhydrotetracyclines. Discard these fractions. The fractions after the first yellow band contain the 4-epianhydrotetracycline. Determine the absorbance of each 4-epianhydrotetracycline fraction at the wavelength of maximum absorbance at about 438 nm, with a suitable spectrophotometer, diluting each fraction, if necessary, with chloroform, and using chloroform as the blank. Calculate the quantity, in mg, of 4-epianhydrotetracycline in each fraction by the formula:

$$A \times 2.0 / (1000L),$$

in which *A* is the absorbance, *V* is the volume, in mL, of the fraction taken, *D* is the dilution factor, if the fraction was diluted, and 2.08 is the absorptivity of 4-epianhydrotetracycline at 438 nm. From the sum of the quantities of 4-epianhydrotetracycline found in the fractions, calculate the percentage of 4-epianhydrotetracycline in relation to the tetracycline hydrochloride equivalent contained in the *Test Solution*.

### (231) HEAVY METALS

This test is provided to demonstrate that the content of metallic impurities that are colored by sulfide ion, under the specified test conditions, does not exceed the *Heavy metals* limit specified in the individual monograph in percentage (by weight) of lead in the test substance, as determined by concomitant visual comparison (see *Visual Comparison in the reaction Procedure under Spectrophotometry and Light Scattering* (851)), with a control prepared from a *Standard Lead Solution*. [NOTE—Substances that typically will

respond to this test are lead, mercury, bismuth, arsenic, tin, cadmium, silver, copper, and molybdenum.]

Determine the amount of heavy metals by *Method I*, otherwise specified in the individual monograph. *Method I* is used for substances that yield clear, colorless preparations under the test conditions. *Method II* is used for substances that do not yield clear, colorless preparations under the test conditions. *Method I*, or for substances that, by virtue of their complexity, interfere with the precipitation of metals by sulfide ion, or with volatile oils. *Method III*, a wet-digestion method, is used in those cases where neither *Method I* nor *Method II* can be used.

#### Special Reagents

**Lead Nitrate Stock Solution**—Dissolve 159.8 mg of lead nitrate in 100 mL of water to which has been added 1 mL of nitric acid. Dilute with water to 1000 mL. Prepare and store this solution in containers free from soluble lead salts.

**Standard Lead Solution**—On the day of use, dilute 10.0 mL of *Lead Nitrate Stock Solution* with water to 100.0 mL. Each mL of *Standard Lead Solution* contains the equivalent of 10 µg of lead. A *comparison solution* prepared on the basis of 100 µg of lead per g of substance being tested contains the equivalent of 1 part of lead per million parts of substance being tested.

#### METHOD I

**pH 3.5 Acetate Buffer**—Dissolve 25.0 g of ammonium acetate in 25 mL of water, and add 38.0 mL of 6 N hydrochloric acid. Adjust, if necessary, with 6 N ammonium hydroxide or 6 N hydrochloric acid to a pH of 3.5; dilute with water to 100 mL, and mix.

**Standard Preparation**—Into a 50-mL color-comparison tube pipet 2 mL of *Standard Lead Solution* (20 µg of Pb), and dilute with water to 25 mL. Using a pH meter or short-range pH indicator paper as external indicator, adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, dilute with water to 40 mL, and mix.

**Test Preparation**—Into a 50-mL color-comparison tube pipet 2 mL of the solution prepared for the test as directed in the individual monograph; or, using the designated volume of acid where specified in the individual monograph, dissolve in and dilute with water to 25 mL the quantity, in g, of the substance to be tested, as calculated by the formula:

$$2.0 / (1000L),$$

in which *L* is the *Heavy metals* limit, as a percentage. Using a pH meter or short-range pH indicator paper as external indicator, adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, dilute with water to 40 mL, and mix.

**Monitor Preparation**—Into a third 50-mL color-comparison tube place 25 mL of a solution prepared as directed for *Test Preparation*, and add 2.0 mL of *Standard Lead Solution*. Using a pH meter or short-range pH indicator paper as external indicator, adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, dilute with water to 40 mL, and mix.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, then add 1.2 mL of the designated volume of glycerin base TS, dilute with water to 50 mL, mix, allow to stand 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is equal to or darker than that of the solution from the *Standard Preparation*. [NOTE—If the color of the *Monitor Preparation* is lighter than that of the *Standard Preparation*, use *Method II* instead of *Method I* for the substance being tested.]

In those countries or jurisdictions where thioacetamide cannot be used, add 10 mL of freshly prepared hydrogen sulfide TS to each of the tubes, and stand for 5 minutes, and view downward over a white surface.

**METHOD II**

...method does not recover mercury.

**Acetate Buffer**—Prepare as directed under *Method I*.

**Standard Preparation**—Pipet 4 mL of the *Standard Lead* into a suitable test tube, and add 10 mL of 6 N hydrochloric acid.

**Test Preparation**—Use a quantity, in g, of the substance to be tested, calculated by the formula:

$$2.0/(1000L)$$

in which *L* is the *Heavy metals* limit, as a percentage. Transfer the weighed quantity of the substance to a suitable crucible, add sufficient sulfuric acid to wet the substance, and carefully ignite at a low temperature until thoroughly charred. (The crucible may be loosely covered with a suitable lid during the charring.) Add to the crucible 2 mL of nitric acid and 5 drops of sulfuric acid, and cautiously until white fumes no longer are evolved. Ignite in a muffle furnace, at 500° to 600°, until the carbon is completely burned off (no longer than 2 hours). If carbon remains, allow the residue to cool, add a few drops of sulfuric acid, evaporate to dryness again. Cool, add 5 mL of 6 N hydrochloric acid, cover, and digest on a steam bath for 10 minutes. Cool, and quantitatively transfer the solution to a test tube. Rinse the crucible with a second 5-mL portion of 6 N hydrochloric acid, and transfer the rinsing to the test tube.

**Monitor Preparation**—Pipet 4 mL of the *Standard Lead Solution* into a test tube identical to that used for the *Test Preparation* and add the same quantity of the substance under test that is equal to 10% of the amount required for the *Test Preparation*. Evaporate on a steam bath to dryness. Ignite at the same time, in the same muffle furnace, under the same conditions used for the *Test Preparation*. Cool, add 5 mL of 6 N hydrochloric acid, cover, and digest on a steam bath for 10 minutes. Cool, and quantitatively transfer to a test tube. Rinse the crucible with a second 5-mL portion of 6 N hydrochloric acid, and transfer the rinsing to the test tube.

**Procedure**—Adjust the solution in each of the tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation* with ammonium hydroxide, added cautiously and adjusted to a pH of 9. Cool, and adjust with glacial acetic acid, added cautiously, to a pH of 8, then add 0.5 mL in excess. Using a pH indicator of short-range pH indicator paper as external indicator, check the pH and adjust, if necessary, with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0. Filter, if necessary, washing the filter with a few mL of water, into a 50-mL color-comparison tube, and then dilute with water to 40 mL. Add 2 mL of pH 3.5 *Acetate Buffer*, then add 1.2 mL of thioacetamide-glycerin base TS, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface; the color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is equal to or darker than that of the solution from the *Standard Preparation*. [NOTE—If the color of the solution from the *Monitor Preparation* is lighter than that of the solution from the *Standard Preparation*, proceed as directed for *Method III* for the substance being tested.]

**METHOD III**

**3.5 Acetate Buffer**—Prepare as directed under *Method I*.

**Standard Preparation**—Transfer a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid to a clean, dry, 100-mL Kjeldahl flask, and add a further volume of nitric acid equal to the incremental volume of nitric acid added to the *Test Preparation*. Heat the solution to the production of dense, white fumes; cool; cautiously add 10 mL of water, and, if hydrogen peroxide was used in treating the *Test Preparation*, add a volume of 30 percent hydrogen peroxide equal to the amount for the substance being tested. Boil gently to the production of dense, white fumes. Again cool, cautiously add 5 mL of water, and boil gently to the production of dense, white fumes and to a volume of 2 to 3 mL. Cool, dilute cautiously with a few mL of water, and add 2 mL of *Standard Lead Solution* (20 µg of Pb), and mix. Transfer to a 50-mL color-comparison tube, rinse the flask with water, and transfer the rinsing to the tube until the volume is 25 mL, and mix.

**Test Preparation**—Unless otherwise indicated in the individual monograph, use a quantity, in g, of the substance to be tested as calculated by the formula:

$$2.0/(1000L)$$

in which *L* is the *Heavy metals* limit, as a percentage.

**If the substance is a solid**—Transfer the weighed quantity of the test substance to a clean, dry, 100-mL Kjeldahl flask. [NOTE—A 300-mL flask may be used if the reaction foams excessively.] Clamp the flask at an angle of 45°, and add a sufficient quantity of a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid to moisten the substance thoroughly. Warm gently until the reaction commences, allow the reaction to subside, and add portions of the same acid mixture, heating after each addition, until a total of 18 mL of the acid mixture has been added. Increase the amount of heat, and boil gently until the solution darkens. Cool, add 2 mL of nitric acid, and heat again until the solution darkens. Continue the heating, followed by addition of nitric acid until no further darkening occurs, then heat strongly to the production of dense, white fumes. Cool, cautiously add 5 mL of water, boil gently to the production of dense, white fumes, and continue heating until the volume is reduced to a few mL. Cool, cautiously add 5 mL of water, and examine the color of the solution. If the color is yellow, cautiously add 1 mL of 30 percent hydrogen peroxide, and again evaporate to the production of dense, white fumes and a volume of 2 to 3 mL. If the solution is still yellow, repeat the addition of 5 mL of water and the peroxide treatment. Cool, dilute cautiously with a few mL of water, and rinse into a 50-mL color-comparison tube, taking care that the combined volume does not exceed 25 mL.

**If the substance is a liquid**—Transfer the weighed quantity of the test substance to a clean, dry, 100-mL Kjeldahl flask. [NOTE—A 300-mL flask may be used if the reaction foams excessively.] Clamp the flask at an angle of 45°, and cautiously add a few mL of a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid. Warm gently until the reaction commences, allow the reaction to subside, and proceed as directed for *If the substance is a solid*, beginning with "add portions of the same acid mixture."

**Monitor Preparation**—Proceed with the digestion, using the same amount of sample and the same procedure as directed in the subsection *If the substance is a solid* in the section *Test Preparation*, until the step "Cool, dilute cautiously with a few mL of water." Add 2.0 mL of *Lead Standard Solution* (20 µg of lead), and mix. Transfer to a 50-mL color comparison tube, rinse the flask with water, adding the rinsing to the tube until the volume is 25 mL, and mix.

**Procedure**—Treat the *Test Preparation*, the *Standard Preparation*, and the *Monitor Preparation* as follows. Using a pH meter or short-range pH indicator paper as external indicator, adjust the solution to a pH between 3.0 and 4.0 with ammonium hydroxide (a dilute ammonia solution may be used, if desired, as the specified range is approached), dilute with water to 40 mL, and mix.

To each tube add 2 mL of pH 3.5 *Acetate Buffer*, then add 1.2 mL of thioacetamide-glycerin base TS, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface; the color of the *Test Preparation* is not darker than that of the *Standard Preparation*, and the color of the *Monitor Preparation* is equal to or darker than that of the *Standard Preparation*.

**(241) IRON**

This limit test is provided to demonstrate that the content of iron, in either the ferric or the ferrous form, does not exceed the limit for iron specified in the individual monograph. The determination is made by concomitant visual comparison with a control prepared from a standard iron solution.

**Special Reagents**—

**STANDARD IRON SOLUTION**—Dissolve 863.4 mg of ferric ammonium sulfate [FeNH<sub>4</sub>(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O] in water, add 10 mL of 2 N sulfuric acid, and dilute with water to 100.0 mL. Pipet 10 mL of this solution into a 1000-mL volumetric flask, add 10 mL of 2 N sulfuric acid, dilute with water to volume, and mix; This solution contains the equivalent of 0.01 mg (10 µg) of iron per mL.

## Appendix K

### Truthful and Accurate Statement

**TRUTHFUL AND ACCURATE STATEMENT**

**As Required per 21 CFR 807.87(k)**

I certify that, in my capacity as Senior Vice President of ReGen Biologics, I believe to the best of my knowledge, that all data and information submitted in the premarket notification are truthful and accurate and that no material fact has been omitted.



John Dichiara