

**Module 2**  
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### **PART 3. NON-CLINICAL INVESTIGATIONS OF THE DEVICE**

#### **3.1 Summary of the Non-clinical Laboratory Studies**

Extensive studies were conducted to characterize the chemical, physical and biological properties of Hylaform (hylan B gel). These studies include a large number of nonclinical laboratory studies to examine biological and safety properties. Under the conditions of the *in vitro* and *in vivo* biological tests, Hylaform was found to be a non-irritant, non-pyrogenic, non-immunogenic, non-carcinogenic, non-hemolytic and non-cytotoxic.

Hylan B gel and its precursor, hylan A, have been characterized chemically and physically by extensive elemental, chemical, spectroscopic and enzymatic analyses. From this testing it was established that hylan B gel (Hylaform) is a clear, transparent gel with rheological properties appropriate for the mode of action of Hylaform. It is also established that hylan B polymer concentration in the gel is 4.5 – 6.5 mg/ml, with osmolality of 290 – 330 mOsm, and median particle size of approximately 500 µm. Chemical analyses of hylan A demonstrate that this slightly modified form of hyaluronic acid is, in essence, chemically equivalent to native hyaluronan and the basic structure of hyaluronan is retained in hylan A. Hylan B is a cross-linked derivative of hylan A in which the basic structure of the polymer backbone is retained, and thus has similar properties to native hyaluronan. Enzymatic studies have shown that hylan B is similar to native hyaluronan because pendant groups and cross-links do not interfere with the enzyme's ability to recognize hylan B as a substrate, allowing the product to be eliminated from the body by the normal metabolic routes.

The useful life of Hylaform has been determined through a broad range of studies that were conducted to ensure that controlled conditions under which the material is manufactured and packaged are more than adequate to maintain the characteristics established for the safe and effective use of the device. These studies have established that Hylaform (hylan B gel) is a sterile (SAL 10<sup>-6</sup>), single use device and maintains its essential characteristics for up to 24 months at 30° C.

The results of testing are summarized below and reviewed in detail in Sections 3.2, 3.3, and 3.4.

### 3.1.1 Biological Testing

Hylaform (hylan B gel) was studied extensively in a large number of nonclinical laboratory studies to characterize biological properties and ensure safety. In all toxicity studies of hylan B, the concentrations (masses) of the polymers used were comparable to or exceeded the anticipated clinical use. Concentrations (masses) higher than those intended for clinical use were used in some studies to enhance any potential toxicity. Reference is made to hylan B gel throughout this document as hylan gel, hylan gel slurry, and hylan B. Materials referred to with these names are one material, i.e. hylan B gel. The studies conducted on hylan B and Hylaform include:

#### Short Term Biological Tests

- Irritation Tests
- Sensitization and Immunogenicity Assays
- Cytotoxicity
- Acute Systemic Toxicity
- Hemocompatibility
- Pyrogenicity
- Implantation
- Mutagenicity

#### Long Term Biological Tests

- Subchronic Toxicity
- Chronic Toxicity and Carcinogenicity
- Reproductive Study

#### Pharmacokinetics and Pharmacodynamics

The biological studies conducted on Hylaform are consistent with those recommended under ANSI/AAMI ISO 10993-1:1994 (Biological Evaluation of medical devices –PART 1: Guidance on selection of tests) and FDA guidance document, G95-1 for a tissue

implant with a contact duration of greater than 30 days. The results of this testing are summarized below and reviewed in detail in Section 3.2.1 Biological Testing.

**Irritation:** These tests were conducted to evaluate the potential of hylan B gel to cause irritation in rabbits. Two studies were conducted for this purpose, an intracutaneous toxicity study and subcutaneous implantation study. Under the conditions of these studies, hylan B did not produce any evidence of causing local irritation.

**Sensitization and Immunogenicity:** To evaluate humoral and cellular immunity of hylan B, Collagen I (Zyplast®) or Collagen II (Zyderm®) in the presence and absence of adjuvant (Freunds), rabbits were administered the test articles via repeated intramuscular injections. Each rabbit also received an intradermal injection of the corresponding test article as a challenge dose at two later time points in the study. The injection site was monitored for erythema and edema. Blood samples were collected at various time points and analyzed for the presence of antibodies to the test article using the ELISA procedure. At the end of the study, a complete gross necropsy was performed on all rabbits with a complete histopathological examination of the test article and challenge injection sites.

Additionally, two studies were conducted to evaluate the potential of hylan B gel to be a dermal sensitizer in the guinea pig model. The two studies had similar methodologies – the main difference was that hylan B gel was enzymatically degraded in one study and not degraded in the other. Test material was administered in two induction phases, first intradermally, then topically. This was followed by a challenge application of test article using a Hill Top Chamber. In the study with undegraded hylan B gel, another challenge dose was administered later at a different topical site. The dermal reaction was evaluated according to Draize criteria.

Under the conditions of these tests, hylan B gel without adjuvant did not elicit a humoral or cellular response nor did it show potential to cause dermal sensitization.

**Cytotoxicity:** Testing was conducted *in vitro* to assess the potential of hylan B to produce cytotoxicity in the fibroblast cell culture model. Two *in vitro* studies were conducted using the L929 mouse fibroblast cell line. Under the conditions of these tests, hylan B did not produce any evidence of causing cell lysis or cytotoxicity.

**Acute systemic toxicity:** Testing was done in a study designed to evaluate the potential of hylan B to cause systemic toxicity in the mouse. A large dose of test article was

administered via intraperitoneal injection. Each study animal was observed for signs of adverse reactions. Hylan B was not systemically toxic to mice under the conditions of this study.

**Hemocompatibility:** Testing was conducted to evaluate the potential of hylan B to cause hemolysis using the USP direct contact method *in vitro*. Hylan B gel was not hemolytic under test conditions.

**Pyrogenicity:** Testing was conducted to evaluate the potential of hylan B to produce a pyrogenic effect in a rabbit model. In the rabbit pyrogen test (USP test method for material mediated pyrogenicity), hylan B was injected intravenously into NZW rabbits. The body temperature was measured over time. Under the conditions of these studies, hylan B was determined to be nonpyrogenic.

**Tissue Implantation:** Seven-day and 30-day intramuscular implantation studies were conducted to evaluate the potential of hylan B to cause irritation or toxicity when implanted in living tissue of the rabbit. Hylan B produced macroscopic responses that were comparable to a negative control implant material.

**Mutagenicity:** Hylan B was evaluated for mutagenic and clastogenic potential in a series of *in vitro* test procedures, which included the AMES assay, a chromosome aberration assay in Chinese hamster ovary (CHO) cells, a CHO/hypoxanthine guanine phosphoribosyl transferase (HGPRT) mutation assay, and a transformation assay. In these studies hylan B was found not to be mutagenic or clastogenic.

**Subchronic Toxicity:** Studies were conducted to evaluate the potential toxicity of hylan B in the guinea pig and rabbit models.

Three groups of guinea pigs were injected intraperitoneally with hylan B, ultrasound degraded hylan B, or with buffer (control), one injection per week for 2 weeks. One week after the second injection (the guinea pigs were followed for a total of 3 weeks), blood was drawn, and the animals were sacrificed and necropsied. Hylan B, when given in two intraperitoneal injections, produced no significant changes in body weight, weight gain or organ weights. Hematology and clinical chemistry comparisons were equivalent in hylan B and buffer treated groups. No treatment-related gross or histomorphological changes were observed.

Rabbits received intramuscular injections of hylan B and collagen (Zyderm II® and Zyplast®) with and without Freund's adjuvant at six time points. Adjuvant was used to enhance any immunotoxic effects. Rabbits were euthanized 12 weeks after the first injection. Based on the results of this study, it was concluded that repeated injection of hylan B at a total concentration > 30 times that proposed for clinical use, is not locally or systemically toxic to rabbits.

**Chronic Toxicity:** This study was conducted to evaluate the potential of hylan B to cause toxicity or carcinogenicity in the rat. Test animals were exposed to a large volume of hylan B, administered subcutaneously, for a 12-month period. Under the conditions of this study, subcutaneous administration of hylan B to rats, at a dose over 500 times the proposed clinical dose, was not associated with local or systemic toxicity, nor was it associated with carcinogenic potential or with development of neoplastic lesions.

**Reproduction:** A General Reproduction Study was conducted retrospectively in a primate model, the owl monkey. The primates were used to assess male and female parameters and the viability, external morphology, growth, and general functional development of the offspring. Observations were compiled from 48 male and 57 female owl monkeys in the breeding colony at the Testing Facility and included monkeys used in Basic Exploratory Studies of various hylan test articles and monkeys used in routine toxicity testing using the Monkey Eye Test.

Based on the results of this retrospective primate study, intravitreal injection of hylan B did not affect mating or fertility of these male animals or the viability, external morphology, or growth of the offspring. Similarly, intravitreal injection of hylan B did not affect female reproductive performance or the viability, external morphology, or growth of their offspring. Results from this study indicated that hylan B does not adversely affect reproduction and that it is not a developmental hazard to the offspring.

### **Pharmacokinetics**

The pharmacokinetic characteristics of hylan B have been evaluated in studies in rats and guinea pigs. Radiolabeled hylan B was prepared according to the standard procedures and conditions used in manufacturing of hylan B, with the exception that the process was scaled down proportionately. Radiolabeled reagent was introduced during the preparation process and became covalently incorporated into the hylan B. Analysis of the

final radiolabeled hylan B showed that it was equivalent to hylan B produced during the standard manufacturing process and fulfilled all the specifications of hylan B.

Intradermal residence time of hylan B was assessed in guinea pigs. The intradermal tissue compartment represents the proposed clinical route of administration for hylan B in the dermal tissue. Blood elimination kinetics and tissue distribution of hylan B was studied in female Sprague Dawley rats. The results from these studies indicate that intradermal administration of [<sup>3</sup>H]-hylan B, at an amount that exceeds that estimated for clinical use, results in the formation of a stable biocompatible implant (90% present after 4 weeks) that is not associated with local or systemic toxicity, and that degraded hylan B appears to follow the known blood elimination pathway for hyaluronan and does not exhibit hepatotoxic properties after direct intravenous injection.

### **Pharmacodynamics**

Guinea pig studies were conducted to assess the behavior and long-term tissue effects of intradermally and subdermally injected hylan B as a potential soft tissue augmentation material and to evaluate the residence time, systemic distribution, and local tissue reaction following intradermal administration of [<sup>14</sup>C]-hylan B gel. Results from these studies indicate that hylan B gel is biologically compatible and relatively stable (present at week 52) when implanted into the dermal and subdermal tissue of guinea pigs, and does not produce any remarkable local tissue reaction or toxicity.

#### **3.1.2 Product Testing**

Hylaform (hylan B gel) and its precursor hylan A have been characterized by extensive elemental, chemical, spectroscopic and enzymatic analyses. Hylan B gel is prepared by chemical cross-linking of hylan A using vinyl sulfone. The rheological characteristics of hylan B are critical for the mode of action of Hylaform and have been investigated extensively. The chemistry and physical properties are described in detail in section 3.3 Product Testing.

Analyses of hylan B gel have shown that the hylan B polymer concentration is 4.5-6.5 mg/ml, in a hydration fluid of 0.15 M NaCl. The osmolality of hylan gel is approximately 290-330 mOsm. The median size of particles in hylan gel slurry is approximately 500 µm. The level of heavy metals is less than 2ppm. Hylan B gel exhibits dynamic rheological properties typical of polymer gels, with the elastic properties dominating the

viscous properties at all accessible frequencies. Hylan B can be completely degraded by mammalian hyaluronidase, with production of low molecular weight oligosaccharides. Hylan B is also susceptible to degradation by oxygen-derived free radicals and mechanical forces. The rate of degradation is relatively slow in healthy skin because healthy skin is not subjected to high mechanical stress and strain or sustained inflammatory processes that produce oxygen-derived free radicals. The rate of hyaluronan turnover by hyaluronidase activity is also relatively slow.

Hylan A is a modified form of the naturally occurring glycosaminoglycan, hyaluronan. Treatment with a dilute formaldehyde solution prior to extraction from hyaluronan-containing tissues results in cross-links between polysaccharide chains of hylan involving formaldehyde and reactive groups of a specific protein.

Analyses have shown that the elemental content of hylan A closely corresponds to pure hyaluronan and that the basic structure of hyaluronan is retained in hylan A. Due to cross-linking, the average molecular weight of hylan A is 6 to 7 million daltons, which is significantly higher than hyaluronan.

### **3.1.3 Useful Life**

The useful life of Hylaform has been determined through extensive studies to ensure that controlled conditions during device manufacture and packaging are more than adequate to maintain the characteristics established for the safe and effective use of the device.

Controls begin with the manufacturing process where Hylaform is produced and filled aseptically and is terminally sterilized using a validated autoclave cycle. In-process controls have been established to ensure a sterility assurance level (SAL) of  $10^{-6}$  for the final product Hylaform.

Packaging further ensures the integrity of the product. An aerosol challenge study demonstrated 100% container closure properties. The proposed Hylaform commercial unit package underwent ship testing procedures. Acceptance criteria were final release specifications for the Hylaform final product. Based upon data and information collected during temperature conditioning, vibration and drop testing, the finished device packaging for Hylaform provides adequate physical protection to prevent damage to the syringe and inner box, and the primary package provides sufficient protection to prevent the ingress of microorganisms.

Finally, stability studies have established that Hylaform is stable at 30° C for up to 24 months. This is based on the product meeting all final release specifications at the 24-month time point. Stability studies are ongoing to support a proposed shelf-life extension to 36 months.

### 3.2 Biological Testing

Biological *in vitro* and *in vivo* studies were conducted to evaluate the potential of hylan B to cause irritation and sensitization. The immunogenicity of hylan B was also examined. Biological testing encompasses biocompatibility, pharmacokinetics, and pharmacodynamics. **Table 3-1** and **Table 3-2** summarize all the testing reviewed in this section. **Table 3-3** shows Hylan B gel biocompatibility testing compliance with Blue Book Memorandum #G95-1. These tables are followed by discussion for each test. Formal summary reports for each study are included in **Section 3.2.5**.

The formal summary reports of the nonclinical laboratory studies are organized into short-term studies (single dose and *in vitro* experiments), long-term studies and repeat-dose studies, and pharmacokinetics and pharmacodynamics, as suggested in International Standard ISO 10993. The full reports are located in the archives at Genzyme Biosurgery in the care of the Document Management Group. All or part of the information contained herein has previously been submitted in PMA 950028 and IDE G000315.

In all toxicity studies of hylan B, the mass of the polymer used was comparable to or exceeded the anticipated clinical use. Masses higher than those intended for clinical use were used in some studies to enhance any potential toxicity. In order to interpret the results of nonclinical studies relative to potential human exposure, it is important to meaningfully relate the animal exposure in nonclinical studies to human exposure in clinical trials. The animal exposures in these studies are compared to human exposure based on a mg/kg basis (mg of polymer per kg of body weight). Doses in the animal toxicity studies ranged from 0.93 mg/kg to 280 mg/kg. The anticipated human clinical dose is 2 ml/60 kg of body weight or 0.17 mg/kg.

US Food and Drug Administration regulations for Good Laboratory Practices (Good Laboratory Practices Regulations; Final Rule as cited in 21 CFR Part 58) were the basis for GLP compliance for nonclinical laboratory studies that are critical for safety evaluation. Those studies with deviations from the GLP regulations are noted as non-GLP studies. Quality assurance findings are documented and were provided to the study director and testing facility management. Deviations are documented in the full reports, in compliance with Good Laboratory Practices.

Other studies, designated as Basic Exploratory Studies (conducted primarily to elucidate biological properties or to supplement existing experiments), were conducted in accordance to internal standards but were not in strict compliance with GLP procedures. The Basic Exploratory Studies are summarized in section 3.2.4 immediately following Pharmacodynamics.

**Table 3-1**  
**Summary of Nonclinical Studies: Iso Biocompatibility Tests Performed under GLP**

| Study Number<br>Title                                                                                                                       | Study<br>Duration | No. of Animals/<br>Species                                         | Dose/Route of<br>Administration<br>Anticipated Human Dose =<br>0.03 ml/kg or 0.17 mg/kg | Study Results                                                                                                       |
|---------------------------------------------------------------------------------------------------------------------------------------------|-------------------|--------------------------------------------------------------------|-----------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------|
| <b>SHORT-TERM BIOLOGICAL TESTS</b>                                                                                                          |                   |                                                                    |                                                                                         |                                                                                                                     |
| <b>Irritation Tests</b>                                                                                                                     |                   |                                                                    |                                                                                         |                                                                                                                     |
| <b>BXR 25204-F-I GLP</b><br>Intracutaneous Toxicity Study in the Rabbit<br>NAmSA # 93T-05713-00                                             | 24, 48, 72 hours  | 2 NZW rabbits, avg. weight unspecified (estimated weight = 2.5 kg) | 0.2 mL hylan B per site ID (intradermal) (~0.08 mL/kg) 5 sites/animal                   | No evidence of significant irritation or local toxicity                                                             |
| <b>BXR 23004-I GLP</b><br>Subcutaneous Implantation Study (With Histopathology) in the Rabbit (2 Day)<br>NAmSA # 94T-01840-00, 94T-01840-01 | 2 days            | 8 NZW rabbits (4 treated, 4 control) minimum weight/animal 2.5 kg  | 0.2 mL hylan B per site ID (0.08 mL/kg) 4 sites/animal                                  | Macro- and microscopic reactions were not significant as compared with reactions caused by control article (saline) |

Table 3-1  
 Summary of Nonclinical Studies: Iso Biocompatibility Tests Performed under GLP (continued)

| Study Number<br>Title                                                                                                                                                                              | Study<br>Duration   | No. of Animals/<br>Species                                                                    | Dose/Route of<br>Administration<br>Anticipated Human Dose =<br>0.03 ml/kg or 0.17 mg/kg                                                                                                                                                                                                                                         | Study Results                                                                                    |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|-----------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| <b>Sensitization and Immunogenicity Tests</b>                                                                                                                                                      |                     |                                                                                               |                                                                                                                                                                                                                                                                                                                                 |                                                                                                  |
| <b>BXR 23006-I GLP</b><br>Immunization and Subchronic<br>Intramuscular Toxicity Study of Hylan B<br>(hylan gel), Collagen I (Zyplast®) or<br>Collagen II (Zyderm II®) in Rabbits<br>TSI # 005-0001 | 13 weeks            | NZW Rabbits<br>20<br>20<br>20<br>20<br>20<br>10<br>10<br>animal weight 2-4<br>kg each         | IM (intramuscular)<br>material (mg/kg)<br>Hylan gel + adjuvant (0.93)<br>Collagen I + adjuvant (1.17)<br>Collagen II + adjuvant (1.08)<br>Hylan gel (0.93)<br>Collagen I (1.17)<br>Collagen II (1.08)<br>Collagen I (5.83)<br>Collagen II (5.42)<br>6 injections/animal plus 2 ID<br>challenge doses of 0.01 cc<br>test article | Hylan B without<br>adjuvant = negligible humoral or<br>cellular response                         |
| <b>BXR 20501-I GLP</b><br>Guinea Pig Dermal Sensitization Test<br>(Maximization Study Using Degraded<br>Hylan Gel)<br>NAmSA # 87T-157370-00                                                        | 3 weeks + 5<br>days | 15 albino Hartley<br>guinea pigs: 10<br>treated, 5 control<br>animal weight<br>348-448 g each | 0.1 mL ID<br>0.3 mL topical<br>0.3 mL topical challenge @<br>14 days                                                                                                                                                                                                                                                            | Hylan B did not exhibit any potential<br>to cause dermal sensitization                           |
| <b>BXR 20008-I GLP</b><br>Delayed Contact Sensitization Study (A<br>Maximization Method) in the Guinea Pig<br>NAmSA # 95T-09406-00                                                                 | 4 weeks + 5<br>days | 15 albino Hartley<br>guinea pigs: 10<br>treated, 5 control<br>animal weight<br>333-416 g each | 0.1 mL ID<br>0.3 mL topical<br>0.3 mL topical challenge @<br>14 days & 14 days + 96<br>hours                                                                                                                                                                                                                                    | Hylan B gel showed no significant<br>evidence of causing delayed dermal<br>contact sensitization |

Table 3-1  
 Summary of Nonclinical Studies: Iso Biocompatibility Tests Performed under GLP (continued)

| Study Number<br>Title                                                                                                                                | Study<br>Duration   | No. of Animals/<br>Species                                                | Dose/Route of<br>Administration<br>Anticipated Human Dose =<br>0.03 ml/kg or 0.17 mg/kg | Study Results                                                                                                                                   |
|------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|---------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Cytotoxicity Tests</b>                                                                                                                            |                     |                                                                           |                                                                                         |                                                                                                                                                 |
| <b>BXR 25203-F-I GLP</b><br><i>In Vitro</i> Cytotoxicity (MEM Elution Method) Study in the L929 Mouse Fibroblast Cell Line<br>NAmSA # 93T-05712-00   | 72 hours incubation | N/A                                                                       | 4 g test article/20 mL of vehicle                                                       | Hylan B did not produce any evidence of cell lysis or cytotoxicity                                                                              |
| <b>BXR 23005-I GLP</b><br><i>In Vitro</i> Cytotoxicity Study (Agarose Overlay Method) in the L929 Mouse Fibroblast Cell Line<br>NAmSA # 94T-01840-00 | 24 hours incubation | N/A                                                                       | 0.1 mL aliquot dosed to filter disc applied to agarose surface                          | Hylan B did not produce any evidence of cell lysis or cytotoxicity                                                                              |
| <b>Acute Systemic Toxicity Tests</b>                                                                                                                 |                     |                                                                           |                                                                                         |                                                                                                                                                 |
| <b>BXR 25200-F-I GLP -</b><br>Systemic Toxicity Study in Mice<br>NAmSA # 93T-05713-00                                                                | 72 hours            | 20 non-Swiss albino CFI-derived strain mice<br>animal weight 17-23 g each | 50 mL/kg<br>280 mg/kg<br>IV/IP injection<br>(intravenous/intraperitoneal)               | Hylan B injected via the IP route was not systemically toxic to mice. IV route was judged unacceptable for the gel material used in this study. |

Table 3-1  
 Summary of Nonclinical Studies: Iso Biocompatibility Tests Performed under GLP (continued)

| Study Number<br>Title                                                                                          | Study<br>Duration | No. of Animals/<br>Species                       | Dose/Route of<br>Administration<br>Anticipated Human Dose =<br>0.03 ml/kg or 0.17 mg/kg | Study Results                             |
|----------------------------------------------------------------------------------------------------------------|-------------------|--------------------------------------------------|-----------------------------------------------------------------------------------------|-------------------------------------------|
| <b>Hemocompatibility and Hemolysis Tests</b>                                                                   |                   |                                                  |                                                                                         |                                           |
| <b>BXR 25202-F-I GLP</b><br><i>In Vitro</i> Hemolysis Study<br>(Direct Contact Method)<br>NAmsA # 93T-05712-00 | N/A               | N/A                                              | Hylan B suspended in 0.9% saline solution added to rabbit blood                         | Hylan B gel was not hemolytic             |
| <b>Pyrogenicity Tests</b>                                                                                      |                   |                                                  |                                                                                         |                                           |
| <b>BXR 20001-I GLP</b><br>USP Rabbit Pyrogen Study<br>NAmsA 94T-12580-00                                       | 3 hours           | 3 albino NZW rabbits<br>animal weight 2.8-3.2 kg | 10 mL/kg of 50:1 diluted hylan B gel IV injection                                       | Hylan B was determined to be nonpyrogenic |
| <b>BXR 20002-I GLP</b><br>USP Rabbit Pyrogen Study<br>NAmsA 94T-12581-00                                       | 3 hours           | 3 albino NZW rabbits<br>animal weight 3.0-3.3 kg | 10 mL/kg of 50:1 diluted hylan B gel IV injection                                       | Hylan B was determined to be nonpyrogenic |
| <b>BXR 20003-I GLP</b><br>USP Rabbit Pyrogen Study<br>NAmsA 94T-12582-00                                       | 3 hours           | 3 albino NZW rabbits<br>animal weight 2.6-3.4 kg | 10 mL/kg of 50:1 diluted hylan B gel IV injection                                       | Hylan B was determined to be nonpyrogenic |

Table 3-1  
 Summary of Nonclinical Studies: Iso Biocompatibility Tests Performed under GLP (continued)

| Study Number<br>Title                                                                                                           | Study<br>Duration | No. of Animals/<br>Species                          | Dose/Route of<br>Administration<br>Anticipated Human Dose =<br>0.03 ml/kg or 0.17 mg/kg | Study Results                                                                                                                                                                              |
|---------------------------------------------------------------------------------------------------------------------------------|-------------------|-----------------------------------------------------|-----------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Implantation Tests</b>                                                                                                       |                   |                                                     |                                                                                         |                                                                                                                                                                                            |
| <b>BXR 23003-I GLP</b><br>Muscle Implantation Study (With Histopathology) in the Rabbit<br>NAmSA #94T-01840-00, 94T-01840-01    | 7 Days            | 2 NZW Rabbits<br>minimum<br>weight/animal 2.5<br>kg | 0.2 mL injected IM at each<br>of 4 sites/rabbit<br>(0.32 mL/kg, 1.63 mg/kg)             | Macroscopically produced a response comparable to negative control<br>Microscopically, hylan B was classified as a nonirritant when compared with USP negative control (hylan B score = 0) |
| <b>BXR 25201-F-I GLP</b><br>USP Muscle Implantation Study (With Histopathology) in the Rabbit (7 Days)<br>NAmSA # 93T-05714-00  | 7 Days            | 2 NZW rabbits<br>minimum<br>weight/animal 2.5<br>kg | 0.2 mL injected IM at each<br>of 4 sites/rabbit<br>(0.32 mL/kg, 1.8 mg/kg)              | Macroscopically produced a response comparable to negative control<br>Microscopically, hylan B was classified as a slight irritant (hylan B score = 2)                                     |
| <b>BXR 25205-F-I GLP</b><br>USP Muscle Implantation Study (With Histopathology) in the Rabbit (30 Days)<br>NAmSA # 93T-05715-00 | 30 Days           | 2 NZW rabbits<br>minimum<br>weight/animal 2.5<br>kg | 0.2 mL injected IM at each<br>of 4 sites/rabbit<br>(0.32 mL/kg, 1.8 mg/kg)              | Macroscopically produced a response comparable to negative control<br>Microscopically, hylan B was classified as a nonirritant                                                             |

Table 3-1  
 Summary of Nonclinical Studies: Iso Biocompatibility Tests Performed under GLP (continued)

| Study Number<br>Title                                                                                                                                                                              | Study<br>Duration                                       | No. of<br>Animals/<br>Species | Dose/Route of Administration<br>Anticipated Human Dose =<br>0.03 ml/kg or 0.17 mg/kg | Study Results                                                                                      |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|-------------------------------|--------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------|
| <b>Mutagenicity Tests</b>                                                                                                                                                                          |                                                         |                               |                                                                                      |                                                                                                    |
| <b>BXR 20202-F-I GLP</b><br>Ames Mutagenicity Test of Degraded Hylan Gel<br>NAmSA Lab 87T-15737-00                                                                                                 | 48 hours                                                | N/A                           | 0.0001 to 0.1 mL/plate, 5 doses                                                      | No mutagenic activity detected in assay at any concentration in activated and nonactivated systems |
| <b>BXR 20201-F-I GLP</b><br>Ames Mutagenicity Test of Hylan Gel<br>NAmSA Lab 87T-15738-00                                                                                                          | 48 hours                                                | N/A                           | 0.0001 to 0.1 mL/plate, 5 doses                                                      | No mutagenic activity detected in assay at any concentration in activated and nonactivated systems |
| <b>BXR 23000-I GLP</b><br>Test For Chemical Induction of Gene Mutation at the HGPRT Locus in Cultured Chinese Hamster Ovary (CHO) Cells With and Without Metabolic Activation<br>SITEK # 0265-2510 | 4 hours exposure<br>7 days after experiment to evaluate | N/A                           | 5 to 500 µL/mL (activated)<br>0.05 to 1.0 µL/mL (nonactivated)                       | Hylan B shown to be nonmutagenic and nonclastogenic                                                |

Table 3-1  
 Summary of Nonclinical Studies: Iso Biocompatibility Tests Performed under GLP (continued)

| Study Number<br>Title                                                                                                                                                                                                                                                     | Study Duration                                               | No. of Animals/<br>Species                  | Dose/Route of Administration<br>Anticipated Human Dose =<br>0.03 ml/kg or 0.17 mg/kg | Study Results                                                                                                                                       |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------|---------------------------------------------|--------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>BXR 23001-I GLP</b><br>Test For Chemical Induction of Chromosome Aberration in Cultured Chinese Hamster Ovary (CHO) Cells With and Without Metabolic Activation<br>SITEK # 0265-3113                                                                                   | 20 hours activated;<br>34 hours nonactivated                 | N/A                                         | Up to 500 µL/mL<br>0.50 mL (2.5 mg/mL or 50% v/v)                                    | No chromosomal aberrations in cultured CHO cells either in the presence or absence of metabolizing system                                           |
| <b>BXR 23002-I GLP</b><br>Test For Chemical Induction of Morphological Cell Transformation in Cultured BALB/C-3T3 Cells With and Without Metabolic Activation<br>SITEK # 0265-6100                                                                                        | 2 hours exposure then<br>7 days after experiment to evaluate | N/A                                         | Assay at concentrations of<br>1.0, 5.0, 10 and 20 µg/mL                              | Hylan B did not induce cell transformation in BALB/c-3T3 cells                                                                                      |
| <b>LONG-TERM BIOLOGICAL TESTS</b>                                                                                                                                                                                                                                         |                                                              |                                             |                                                                                      |                                                                                                                                                     |
| <b>Subchronic Toxicity Tests</b>                                                                                                                                                                                                                                          |                                                              |                                             |                                                                                      |                                                                                                                                                     |
| <b>BXR 23006-I GLP</b><br>Immunization and Subchronic Intramuscular Toxicity Study of Hylan B (hylan gel), Collagen I (Zyplast®) or Collagen II (Zyderm II®) in Rabbits<br>TSI # 005-0001                                                                                 | 12 weeks                                                     | 40 NZW rabbits<br>animal weight 2-4 kg each | 1 mg/kg IM at each of 6 time points<br>total dose 5 mg/kg                            | Repeated injections of hylan B at total mass greater than 30 times that proposed for clinical use was not locally or systemically toxic to rabbits. |
| Note: Reference is made to hylan B gel throughout this document as hylan gel, hylan gel slurry, hylan B. Materials referred to with these names are one material, i.e., hylan B gel.<br>Ref.: International Standard ISO 10993 (Biological Evaluation of Medical Devices) |                                                              |                                             |                                                                                      |                                                                                                                                                     |

**Table 3-2**  
**Summary of Nonclinical Studies: Iso Biocompatibility Tests NOT Performed under GLP\***

\*Quality Assurance identified deviations from GLP and these are documented in the full study reports.

| Study Number<br>Title                                                                                                                                                                                                                                                                     | Study<br>Duration | No. of Animals/<br>Species                                                              | Dose/Route of<br>Administration<br>Anticipated Human Dose =<br>0.03 ml/kg or 0.17 mg/kg | Study Results                                                  |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------|-----------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|----------------------------------------------------------------|
| <b>LONG TERM BIOLOGICAL TESTS</b>                                                                                                                                                                                                                                                         |                   |                                                                                         |                                                                                         |                                                                |
| <b>Subchronic Toxicity Tests</b>                                                                                                                                                                                                                                                          |                   |                                                                                         |                                                                                         |                                                                |
| <b>BXR 25305-F-I non-GLP</b><br>Subchronic Two-week Intraperitoneal Toxicity Study on Hylan Gel in Male Guinea Pigs [Effect of Repeated Intraperitoneal Injections of Hylan Gel or Degraded Hylan Gel (2mg/ml) on Blood Parameters and Histology of Selected Tissues in Male Guinea Pigs] | 2 Weeks           | 36 Dunkin Hartley guinea pigs (30 treatment, 6 control)<br>animal weight 297-370 g each | 10 mL/kg = 20 mg/kg IP                                                                  | No treatment-related gross or histopathologic changes observed |

Table 3-2

Summary of Nonclinical Studies: Iso Biocompatibility Tests NOT Performed under GLP\* (continued)

\*Quality Assurance identified deviations from GLP and these are documented in the full study reports.

| Study Number<br>Title                                                                                                                                                                                    | Study<br>Duration               | No. of Animals/<br>Species                                                                           | Dose/Route of<br>Administration<br>Anticipated Human Dose =<br>0.03 ml/kg or 0.17 mg/kg | Study Results                                                                                                                                                                                                                                                            |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Chronic Toxicity and Carcinogenicity Tests</b>                                                                                                                                                        |                                 |                                                                                                      |                                                                                         |                                                                                                                                                                                                                                                                          |
| <b>BXR 25405-F-I non-GLP</b><br>One Year Subcutaneous Toxicity Study of Hylan B in Female Rats<br>Study #13-91                                                                                           | 1 Year                          | 36 Sprague Dawley rats<br>(28 treatment, 8 control)<br>animal weight 200-250 g each                  | 20 mL/kg SC (subcutaneous) (ca. 5 mL per animal)                                        | Hylan B was not associated with local or systemic toxicity, nor was it associated with carcinogenic potential or with development of neoplastic lesions. No treatment-related histopathologic changes. Hylan B still present in injected tissue at 1 year post injection |
| <b>Reproduction Studies Tests</b>                                                                                                                                                                        |                                 |                                                                                                      |                                                                                         |                                                                                                                                                                                                                                                                          |
| <b>BXR 12243-F-I non-GLP</b><br>General Reproduction Study (Segment I) of the Effect of Intraocular/Intra-articular Injection of Hyaluronan, Hylan A, Hylan B and Synvisc® (hylan G-F 20) in Owl Monkeys | Natural life span of the colony | 119 owl monkeys (aotus species)<br>(105 treatment: 48 male, 57 female; 14 control: 7 male, 7 female) | Intravitreal injections                                                                 | Did not affect mating, fertility, viability, external morphology of young or growth of young                                                                                                                                                                             |

Table 3-2  
 Summary of Nonclinical Studies: Iso Biocompatibility Tests NOT Performed under GLP\* (continued)

\*Quality Assurance identified deviations from GLP and these are documented in the full study reports.

| Study Number<br>Title                                                                                                                             | Study<br>Duration | No. of Animals/<br>Species                                          | Dose/Route of<br>Administration<br>Anticipated Human Dose =<br>0.03 ml/kg or 0.17 mg/kg                       | Study Results                                                                                                                                                                                                                                                                                          |
|---------------------------------------------------------------------------------------------------------------------------------------------------|-------------------|---------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Pharmacokinetic Tests</b>                                                                                                                      |                   |                                                                     |                                                                                                               |                                                                                                                                                                                                                                                                                                        |
| <b>BXR 25407-F-I non-GLP</b><br>Intradermal Injection Of [ <sup>3</sup> H]-Hylan B<br>([ <sup>3</sup> H]-hylan gel) in Guinea Pigs<br>Study #1-90 | 4 weeks           | 12 Charles River<br>guinea pigs;<br>animal weight<br>300-350 g each | 0.434 ± 0.247 per site ID<br>4 sites per animal<br>(1.45 mL/kg = 7.5 mg<br>polymer/kg, 50 x proposed<br>dose) | Intradermal administration of [ <sup>3</sup> H]-<br>hylan B forms a stable biocompatible<br>implant (90% present after 4 weeks)<br>which is not associated with local or<br>systemic toxicity.                                                                                                         |
| <b>BXR 25212-F-I</b><br>Distribution of [ <sup>3</sup> H]-Hylan Gel (degraded)<br>in Rats<br>TSI Study #MRI-BMX 339-91-60                         | 24 hours          | 25 Sprague<br>Dawley rats<br>animal weight<br>161-199 g each        | 1 mL IV bolus<br>(27 mg/kg)                                                                                   | Hylan B eliminated from blood<br>>75% within 1 hour via urine with<br>little radioactivity <2.5% recovered<br>from vital organs. No evidence of<br>accumulation in any tissues involved<br>in hyaluronan metabolism. Hylan B<br>appears to follow the same blood<br>elimination pathway as hyaluronan. |

Table 3-2

Summary of Nonclinical Studies: Iso Biocompatibility Tests NOT Performed under GLP\* (continued)

\*Quality Assurance identified deviations from GLP and these are documented in the full study reports.

| Study Number<br>Title                                                                                                                                                                                                                                                                        | Study<br>Duration | No. of Animals/<br>Species                                | Dose/Route of<br>Administration<br>Anticipated Human Dose =<br>0.03 ml/kg or 0.17 mg/kg                       | Study Results                                                                                                                                                                             |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------|-----------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Nonclinical Studies of Efficacy (Pharmacodynamics)</b>                                                                                                                                                                                                                                    |                   |                                                           |                                                                                                               |                                                                                                                                                                                           |
| <b>BXR 25213-I non-GLP</b><br>Six Month Study of Residence Time of [14C]-Hylan B After Intradermal Administration in Female Guinea Pigs                                                                                                                                                      | 6 months          | 18 Hartley guinea pigs;<br>minimum 300 g/animal           | 0.50 mL/animal ID<br>8 sites per animal<br>(1.6 mL/kg/8.8 mg/kg)                                              | t <sub>1/2</sub> = 9.16 months; No evidence of treatment-related effect on dermal tissue injected<br>Histopathology = no detectable inflammation or dermal fibrosis at any injection site |
| <b>BXR 25408-F-I non-GLP</b><br>Intradermal and Subcutaneous Injection of Hylan Gel in Guinea Pigs                                                                                                                                                                                           | 52 weeks          | 16 Hartley guinea pigs<br>animal weight<br>250-350 g each | Injected hylan B or collagen or saline (control)<br>12 sites/animal: 6 sites 0.05 cc ID and 6 sites 0.2 cc SC | Hylan B present at 52 weeks; collagen not there at 52 weeks.<br>Collagen = inflammatory reaction;<br>Hylan B = no inflammatory reaction                                                   |
| <p>Note: Reference is made to hylan B gel throughout this document as hylan gel, hylan gel slurry, hylan B. Materials referred to with these names are one material, i.e., hylan B gel.<br/>           Ref.: International Standard ISO 10993 (Biological Evaluation of Medical Devices)</p> |                   |                                                           |                                                                                                               |                                                                                                                                                                                           |

**Table 3-3**  
**Hylaform® Biocompatibility Tests**  
**Compliance with Blue Book Memorandum #G95-1**  
**Tissue Implant Device Permanent Contact Duration**

| Biological Effect<br>Initial Evaluation<br>Tests | Requirement<br>X = ISO evaluation tests<br>O = May be applicable | Genzyme Test Report                                        |                                                                                                                                                                                                                                                                                                                                                                                                                 |
|--------------------------------------------------|------------------------------------------------------------------|------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cytotoxicity                                     | X                                                                | BXR 25203-F-I<br>BXR 23005-I                               | In Vitro Cytotoxicity (MEM Elution Method) Study in the L929 Mouse Fibroblast Cell Line<br>In Vitro Cytotoxicity Study (Agarose Overlay Method) in the L929 Mouse Fibroblast Cell Line                                                                                                                                                                                                                          |
| Sensitization                                    | X                                                                | BXR 23006-I<br>BXR-20501-I<br>BXR 20008-I                  | Immunization and Subchronic Intramuscular Toxicity Study of Hylan B (hylan gel), Collagen I (Zyplast®) or Collagen II (Zyderm II®) in Rabbits<br>Guinea Pig Dermal Sensitization Test (Maximization Study Using Degraded Hylan Gel)<br>Delayed Contact Sensitization Study (A Maximization Method) in the Guinea Pig                                                                                            |
| Irritation                                       | O                                                                | BXR 25204-F-I<br>BXR 23004-F-I                             | Intracutaneous Toxicity Study in the Rabbit<br>Subcutaneous Implantation Study (With Histopathology) in the Rabbit (2 Day)                                                                                                                                                                                                                                                                                      |
| System Toxicity<br>(Acute)                       | O                                                                | BXR 25200-F-I<br>BXR 20001-I<br>BXR 20002-I<br>BXR 20003-I | Systemic Toxicity Study in Mice<br>USP Rabbit Pyrogen Study<br>USP Rabbit Pyrogen Study<br>USP Rabbit Pyrogen Study                                                                                                                                                                                                                                                                                             |
| Sub-chronic toxicity                             | O                                                                | BXR 23006-I<br>BXR 25305-F-I                               | Immunization and Subchronic Intramuscular Toxicity Study of Hylan B (hylan gel), Collagen I (Zyplast®) or Collagen II (Zyderm II®) in Rabbits<br>Subchronic Two-week Intraperitoneal Toxicity Study on Hylan Gel in Male Guinea Pigs [Effect of Repeated Intraperitoneal Injections of Hylan Gel or Degraded Hylan Gel (2mg/ml) on Blood Parameters and Histology of Selected Tissues in Male Male Guinea Pigs] |

Table 3-3  
 Hylaform® Biocompatibility Tests  
 Compliance with Blue Book Memorandum #G95-1  
 Tissue Implant Device Permanent Contact Duration (continued)

| Biological Effect<br>Initial Evaluation<br>Tests | Requirement<br>X = ISO evaluation tests<br>O = May be applicable | Genzyme Test Report                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|--------------------------------------------------|------------------------------------------------------------------|-----------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Genotoxicity                                     | X                                                                | BXR 20202-F-I<br>BXR 20201-F-I<br>BXR 23000-I<br>BXR 23001-I<br>BXR 23002-I | Ames Mutagenicity Test of Degraded Hylan Gel<br>Ames Mutagenicity Test of Hylan Gel<br>Test For Chemical Induction of Gene Mutation at the HGPRT Locus in Cultured Chinese Hamster Ovary (CHO) Cells With and Without Metabolic Activation<br>Test For Chemical Induction of Chromosome Aberration in Cultured Chinese Hamster Ovary (CHO) Cells With and Without Metabolic Activation<br>Test For Chemical Induction of Morphological Cell Transformation in Cultured BALB/C-3T3 Cells With and Without Metabolic Activation |
| Implantation                                     | X                                                                | BXR 23003-I<br>BXR 25201-F-I<br>BXR 25205-F-I                               | Muscle Implantation Study (With Histopathology) in the Rabbit<br>USP Muscle Implantation Study (With Histopathology) in the Rabbit (7 Days)<br>USP Muscle Implantation Study (With Histopathology) in the Rabbit (30 Days)                                                                                                                                                                                                                                                                                                    |
| Hemocompatibility                                |                                                                  | BXR 25202-F-I                                                               | In Vitro Hemolysis Study (Direct Contact Method)                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| Chronic Toxicity                                 | X                                                                | BXR 25405-F-I                                                               | One Year Subcutaneous Toxicity Study of Hylan B in Female Rats                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Carcinogenicity                                  | X                                                                | BXR 25405-F-I                                                               | One Year Subcutaneous Toxicity Study of Hylan B in Female Rats                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Reproductive<br>Development                      |                                                                  | BXR 12243-F-I                                                               | General Reproduction Study (Segment I) of the Effect of Intraocular/Intra-articular Injection of Hyaluronan, Hylan A, Hylan B and Synvisc® (hylan G-F 20) in Owl Monkeys                                                                                                                                                                                                                                                                                                                                                      |
| Biodegradable                                    |                                                                  | BXR-25407-F-I<br>BXR 25212-F-I<br>BXR 25213-I<br>BXR 25408-F-I              | Intradermal Injection Of [3H]-Hylan B ([3H]-hylan gel) in Guinea Pigs<br>Distribution of [3H]-Hylan Gel (degraded) in Rats<br>Six Month Study of Residence Time of [14C]-Hylan B After Intradermal Administration in Female Guinea Pigs<br>Intradermal and Subcutaneous Injection of Hylan Gel in Guinea Pigs                                                                                                                                                                                                                 |

### **3.2.1 Biocompatibility Narratives**

The biocompatibility of hylan B was evaluated by conducting *in vitro* and *in vivo* studies specifically designed to evaluate irritation, sensitization, and toxicity. Presented here are short-term and long-term and repeated application biological tests.

#### **3.2.1.1 Short Term Biological Tests**

##### **3.2.1.1.1 Irritation**

These tests were conducted to evaluate the potential of hylan B to cause irritation in rabbits. Two studies were conducted for this purpose, an intracutaneous toxicity study and subcutaneous implantation study. Under the conditions of these studies, hylan B did not produce any evidence of causing local irritation.

##### **Intracutaneous Toxicity Study in the Rabbit (BXR-25204-F-I)**

Hylan B was injected into each of five intradermal sites in two rabbits. Injection sites were examined 24, 48, and 72 hours post-injection for erythema and edema. No evidence of significant irritation, or of other signs of local toxicity, was observed in any of the studies. It was concluded that hylan B causes no local short-term irritation in the dermis.

##### **Subcutaneous Implantation Study (with Histopathology) in the Rabbit (2 day) (BXR-23004-I)**

Rabbits were injected with hylan B, four sites in each rabbit. Rabbits were euthanized after 2 days, and macroscopic and microscopic evaluations were performed. Under the conditions of this study, the macroscopic and microscopic reactions were not significant as compared to reactions caused by the control article (saline). Hylan B was classified as a nonirritant.

##### **3.2.1.1.2 Sensitization and Immunogenicity**

The potential irritation, sensitization and immunogenicity of hylan B has been evaluated in the following three nonclinical studies. Under the conditions of these tests, hylan B gel did not elicit a humoral or cellular response nor did it show potential to cause dermal sensitization

**Immunization and Subchronic Intramuscular Toxicity Study of Hylan B (hylan gel), Collagen I (Zyplast®) or Collagen II (Zyderm II®) in Rabbits (BXR-23006-I)**

This study was conducted to evaluate humoral and cellular immunity of hylan B, Collagen I (Zyplast®) or Collagen II (Zyderm®) in the presence and absence of adjuvant following repeated administrations of the test articles.

Male New Zealand White rabbits each received 6 intramuscular injections of test article; days 1, 29, 36, 43, 50, and 57. In groups where adjuvant was used with the test article, Freund's Complete Adjuvant was used for the first injection (day 1) and Freund's Incomplete Adjuvant was used for the subsequent injections. At 43 and 85 days, each rabbit received an intradermal injection of the corresponding test article as a challenge dose. The injection site was monitored for erythema and edema 1 – 3 hours post-injection and at 24 and 48 hours post-injection.

Clinical observations were made weekly and animals were checked twice daily for mortality and moribundity. Body weights were recorded weekly and at necropsy. Blood was collected within 7 days prior to the first injection, then at 1 day prior to day 28, 56, and day 91 – 94 at necropsy. At the end of the study, a complete gross necropsy was performed on all rabbits. Complete histopathological examination included the test article injection site and the challenge injection site.

Two identical sets of serum samples from all study groups and all time points were sent for analysis to two contract laboratories.

No animals died during the study. There were no differences in group mean body weight between treated animals with or without adjuvant. Signs of local irritation were minimal and without clear pattern, and the maximal response observed in hylan gel treated animals was Grade 1 erythema or Grade 1 edema. Collagen treated rabbits produced a maximal response of Grade 2 erythema or Grade 2 edema.

Animals injected repeatedly with hylan B gel without adjuvant did not develop significant serum immune reactivity to the hylan B gel. In 7 of 20 of these animals a titer was detected to avian proteins and/or endotoxin. Hylan gel is prepared from avian tissue and contains low levels of these substances. These study animals exhibited no general

health reactions or significant local reactions, nor did they produce positive reaction to skin testing.

Both hylan B gel and collagen elicited a greater humoral immune response in the presence of adjuvant than in the absence of adjuvant. The results indicate that the antibody response to repeated injections of hylan B gel was adjuvant mediated because in the absence of adjuvant there was no significant titer.

**Guinea Pig Dermal Sensitization Test  
(Maximization Study Using Degraded Hylan Gel)  
(BXR-20501-I)**

This study was conducted to evaluate the potential of hylan B to be a dermal sensitizer in the guinea pig model. In this study, hylan B was evaluated after enzyme treatment with hyaluronidase. Test material was administered intradermally in two rigorous induction phases, first intradermally, then topically. Following the induction phases, each treated animal was challenged with the test article using the nonwoven cotton disk contained in a Hill Top Chamber. At 24, 48, 72 and 96 hours the dermal reaction was evaluated according to Draize criteria. Under the conditions of this test, the hylan B did not exhibit any potential to cause dermal sensitization in the guinea pig.

**Delayed Contact Sensitization Study (A Maximization Method) in the Guinea Pig  
(BXR-20008-I)**

This study was conducted to evaluate the potential for hylan B gel to cause delayed dermal contact sensitization in the guinea pig model. Hylan B gel was not degraded by enzyme treatment for this study. Test material was administered intradermally in two rigorous induction phases. Following the induction phases, each treated animal was challenged with undiluted hylan B using a Hill Top Chamber. At 24, 48, 72, and 96 hours the dermal reaction was evaluated according to Draize criteria. The same animals were rechallenged at a different topical site 4 days after the 96 hour score. Observations for any reactions were conducted at 24, 48, 72, and 96 hours after rechallenge patch removal using the same scoring and evaluation criteria. Under the conditions of this study, the hylan B gel test article showed no significant evidence of causing delayed dermal contact sensitization in the guinea pig.

### 3.2.1.1.3 Cytotoxicity

Testing was conducted *in vitro* to assess the potential of hylan B to produce cytotoxicity in the fibroblast cell culture model. Two *in vitro* studies were conducted using the L929 mouse fibroblast cell line.

#### ***In Vitro* Cytotoxicity Study (MEM Elution Method) in the L929 Mouse Fibroblast Cell Line**

**(BXR-25203-F-I)**

#### ***In Vitro* Cytotoxicity Study (Agarose Overlay Method) in the L929 Mouse Fibroblast Cell Line**

**(BXR 23005-I)**

Under the conditions of these tests, hylan B did not produce any evidence of causing cell lysis or cytotoxicity.

### 3.2.1.1.4 Acute Systemic Toxicity

Acute systemic toxicity was examined in a study designed to evaluate the potential of hylan B to cause systemic toxicity in the mouse. Hylan B was not systemically toxic to mice under the conditions of this study.

#### **Systemic Toxicity Test in Mice**

**(BXR 25200-F-I)**

A large dose of test article was administered via intraperitoneal injection to each study animal. Mice were observed for adverse reactions at 4, 24, 48, and 72 hours. There was no mortality or evidence of toxicity from any hylan B test article injected by this parenteral route. Based on the results of this study, it was concluded that the injected hylan B was not systemically toxic to mice.

### 3.2.1.1.5 Hemocompatibility

#### ***In Vitro* Hemolysis Study (Direct Contact Method)**

**(BXR 25202-F-I)**

Hemocompatibility was evaluated using oxalated rabbit blood. In this procedure, hylan B suspended in 0.9% saline solution is added to rabbit blood and incubated. The resulting

suspension is compared spectrophotometrically against a negative and positive control sample. Hylan B gel was not hemolytic under test conditions.

#### **3.2.1.1.6 Pyrogenicity**

Testing was conducted to evaluate the potential of hylan B to produce a pyrogenic effect in a rabbit model.

##### **USP Rabbit Pyrogen Study (BXR 20001-I)**

##### **USP Rabbit Pyrogen Study (BXR 20002-I)**

##### **USP Rabbit Pyrogen Study (BXR 20003-I)**

In this test for material-mediated pyrogenicity, hylan B (reduced viscosity) was injected intravenously into New Zealand White Rabbits in a single dose of 10 ml/kg of diluted test article (dilution factor was 50). The total rise in body temperature during the three-hour observation period was then determined. Under the conditions of these studies, hylan B was determined to be nonpyrogenic.

#### **3.2.1.1.7 Implantation**

Studies were conducted to evaluate the potential of hylan B to cause irritation or toxicity when implanted in living tissue of the rabbit.

##### **Muscle Implantation Study (with Histopathology) in the Rabbit (BXR 23003-I)**

Hylan B was implanted into the muscle tissue of two New Zealand White rabbits, four test sites per rabbit. Study animals were sacrificed at 7 days post-implantation. Macroscopically, all hylan B implants were found to produce a response comparable to the negative control implant material. Microscopically, hylan B was classified as a nonirritant when compared to the USP negative control; the score for hylan B was 0 (nonirritant).

**USP Muscle Implantation Study (with Histopathology) in the Rabbit (7 Days)  
(BXR 25201-F-I)**

Hylan B was implanted into the muscle tissue of two NZW rabbits, four sites per rabbit. Rabbits were sacrificed 7 days after implantation. Macroscopically, hylan B implants were found to produce a response comparable to the USP negative control. Microscopically, hylan B was classified as a slight irritant when compared to the USP negative control; the score for hylan B was 2 (Scale: 0=nonirritant, 1 – 15= slight irritant, 16 – 30= moderate irritant, >30= severe irritant).

**Muscle Implantation Study with Histopathology in the Rabbit (30 Days)  
(BXR 25205-F-I)**

Hylan B was injected into each of four test sites (muscle) per rabbit. Study animals were sacrificed 30 days after implantation. Macroscopically, hylan B material was found to produce a response comparable to the USP negative control. Microscopically, hylan B was classified as a nonirritant; the score for hylan B was 0 (nonirritant).

**3.2.1.1.8 Mutagenicity**

Hylan B was evaluated for mutagenic and clastogenic potential in a series of test procedures. In none of these procedures was hylan B found to be mutagenic or clastogenic.

**Ames Mutagenicity Test of Degraded Hylan Gel  
(BXR 20202-F-I)**

The study was conducted to evaluate the mutagenic potential of hylan B in the Ames *Salmonella typhimurium* model. Ultrasound degraded hylan B was tested at concentrations designed to produce 0-100% toxicity. There was no mutagenic activity detected in this assay at any concentration of hylan B.

**Ames Mutagenicity Test of Hylan Gel  
(BXR 20201-F-I)**

The study was conducted to evaluate the mutagenic potential of hylan B in the Ames *Salmonella typhimurium* model. There was no mutagenic activity detected in this assay at any concentration of hylan B.

**Test For Chemical Induction of Gene Mutation at the HGPRT Locus in Cultured Chinese Hamster Ovary (CHO) Cells with and without Metabolic Activation (BXR 23000-I)**

The study was conducted to evaluate the potential of hylan B to cause gene mutation at the hypoxanthine guanine phosphoribosyl transferase (HGPRT) locus in cultured Chinese hamster ovary cells (CHO) with and without exogenous metabolic activation. The concentrations of hylan B tested included the standard dose range and a concentration range five times greater than the highest recommended calculation. Six doses were tested in the first assay, four doses were tested in the confirmatory assay. There was no indication of mutagenic activity of hylan B at any concentration and quantity used.

**Test for Chemical Induction of Chromosome Aberration in Cultured Chinese Hamster Ovary (CHO) Cells with and without Metabolic Activation (BXR 23001-I)**

The study was conducted to evaluate the potential of hylan B to induce chromosome aberrations in cultured CHO cells with and without exogenous metabolic activation. There was no evidence that hylan B at any concentration tested (up to 500 µL/ml) was able to induce chromosome aberrations in cultured CHO cells, in the presence or absence of a metabolizing system (rat liver homogenate from Aroclor 1254-induced rats, S-9 activation system).

**Test For Chemical Induction of Morphological Cell Transformation in Cultured BALB/c-3T3 with and without Metabolic Activation (BXR 23002-I)**

This Study was conducted to evaluate the potential carcinogenic effects of hylan B using an *in vitro* test system (Cell Transformation Assay in cultured BALB/c-3T3 cells) with and without exogenous metabolic activation. Toxicity was assessed by measuring the reduction in Relative Cloning Efficiency (RCE). Concentrations of hylan B were selected based on 0-100% cytotoxicity. Hylan B did not induce cell transformation in BALB/c-3T3 cells when tested under the conditions of the study.

### **3.2.1.2 Long-term and Repeated Application Biological Tests**

#### **3.2.1.2.1 Subchronic Toxicity**

Toxicity studies were conducted to evaluate the potential toxicity of hylan B in guinea pig and rabbit models.

#### **Subchronic Two-week Intraperitoneal Toxicity Study on Hylan Gel in Male Guinea Pigs [Effect of Repeated Intraperitoneal Injections of Hylan Gel or Degraded Hylan Gel (2mg/ml) on Blood Parameters and Histology of Selected Tissues in Male Guinea Pigs (BXR-25305-F-I)]**

Three groups of guinea pigs were injected intraperitoneally with hylan B, degraded hylan B, or with buffer (control). A fourth group consisted of untreated animals. A total of two injections (one/week) of hylan B or control were administered. One week after the second injection, blood was drawn, and the animals were sacrificed and necropsied.

Hylan B, when given in two intraperitoneal injections of 20mg/kg, produced no significant changes in body weight, weight gain or organ weights. Hematology and clinical chemistry comparisons were equivalent in hylan B and buffer treated groups. No treatment-related gross or histomorphological changes were observed.

#### **Immunization and Subchronic Toxicity Study of Hylan B (hylan gel), Collagen I (Zyplast®) or Collagen II (Zyderm II®) in Rabbits (BXR-23006-I)**

This study was conducted to evaluate the potential of hylan B and collagen to produce a toxicological response after repeated injections in the rabbit model. Male New Zealand White rabbits received intramuscular injections of hylan B and collagen (Zyderm II and Zyplast) at each of six time points (Weeks 1, 4, 5, 6, 7, and 8). Rabbits were euthanized 12 weeks after the first injection. Six groups of rabbits were injected with 1mg/kg of test article (one test article per group) at each time point. Test articles: hylan B; hylan B with Freund's adjuvant; Zyderm II; Zyderm II with Freund's adjuvant; Zyplast; Zyplast with Freund's adjuvant. Adjuvant was used to enhance any immunotoxic effects. Two additional groups of rabbits were injected with Zyplast (6mg/kg, 6 time points) or Zyderm II (6mg/kg, 6 time points). The immunologic findings are summarized in the immunogenicity section of this application (Refer to Section 3.2.1.1.2). Microscopic

evaluation of injection site tissue (muscle, skin) revealed minimal to minimal-mild inflammation in animals given hylan B without adjuvant and mild to mild-moderate inflammation in animals given hylan B with adjuvant. Animals given hylan B without adjuvant had no other remarkable tissue lesions, with the exception of one rabbit out of 21, which had a single microgranuloma in the lung. Most animals (21/22) given hylan B with adjuvant had microgranulomas in the lungs, and one had microgranulomas in the liver and spleen. These observations were interpreted to be due to systemic translocation of adjuvant moieties. Histological analysis of intradermal injection sites from rabbits treated with Zyplast (6mg/kg) and Zyderm II (6mg/kg), revealed a minimal to moderate inflammatory tissue reaction characterized either by the presence of numerous eosinophils and macrophages or by a minimal encapsulation and minimal macrophage infiltration.

Based on the results of this study, it was concluded that repeated injection of hylan B at a total concentration > 30 times that proposed for clinical use, in not locally or systemically toxic to rabbits.

### **3.2.1.2.2 Chronic Toxicity and Carcinogenicity**

#### **One Year Subcutaneous Toxicity Study of Hylan B in Female Rats (BXR-25405-F-I)**

This study was conducted to evaluate the potential of hylan B to cause toxicity or carcinogenicity in the rat. Test animals were exposed to a large volume of hylan B, administered subcutaneously, for a 12-month period, which represents approximately one-third of their anticipated life span. The study consisted of ten groups of animals: five treatment (hylan B) groups and five control (saline) groups. Gross observations related to animal health (including injection site) were monitored daily. Examination of the injection site (palpation, appearance) was conducted at regular time points throughout the study. Animals were sacrificed at 1 week, 1, 3, 6, and 12 months post implantation. Selected organs and skin tissues were processed for histopathological evaluation.

There were no treatment-related clinical changes in animal health or behavior and there were no detectable clinical signs of local reaction to the injected material (absence of swelling, redness, heat, lesion, etc.). Histopathological evaluation revealed that there were no treatment-related microscopic changes in any organ; all other changes, neoplastic

and nonneoplastic were incidental background lesions that were consistent in incidence and severity with the age of the rats. In skin sections (injection site tissue), an initial inflammatory reaction (observed at 1 week) gradually resolved, and at one year post-treatment, inflammation was not observed in the treatment animals. Hylan B was still present in the injected tissue at one year post-injection.

Under the conditions of this study, subcutaneous administration of hylan B to Sprague Dawley rats, at a dose over 500 times the proposed clinical dose, is not associated with local or systemic toxicity, nor is it associated with carcinogenic potential or with development of neoplastic lesions.

### **3.2.1.2.3      Reproduction Studies**

#### **General Reproduction Study (Segment I) of the Effect of Intraocular/Intra-articular Injection of Hyaluronan, Hylan A, Hylan B, and Synvisc® (hylan G-F 20) in Owl Monkeys (BXR 12243-F-I)**

A General Reproduction Study was conducted retrospectively in a primate model, the owl monkey. The primates were used to assess male and female parameters and the viability, external morphology, growth, and general functional development of the offspring.

Observations were compiled from 48 male and 57 female owl monkeys in the breeding colony at the Testing Facility. These animals were used in Basic Exploratory Studies of various hylan test articles and for routine toxicity testing using the Monkey Eye Test. In each study, the number of injections and the total material injected per animal represented a significant increase over the maximum number of injections and total amount of material intended to be used in clinical situations in humans. The concurrent untreated control group consisted of seven reproductive pairs of male and female owl monkeys.

The incidences of pregnancies, abortions, stillbirths, and early infant deaths in the male and female test groups were not significantly different for those of the concurrent control group and the colony historical incidence.

Based on the results of this retrospective primate study, intravitreal injection of hylan B did not affect mating or fertility of these male animals or the viability, external

morphology, or growth of the offspring. Similarly, intravitreal injection of hylan B did not affect female reproductive performance or the viability, external morphology, or growth of their offspring. Results from this study indicated that hylan B does not adversely affect reproduction and that it is not a developmental hazard to the offspring.

### **3.2.2 Pharmacokinetics**

The pharmacokinetic characteristics of hylan B have been studied in rats and guinea pigs. Radiolabeled hylan B was prepared according to standard procedures and conditions used in manufacturing hylan B, with the exception that the process was scaled down proportionately. Radiolabeled reagent was introduced during the preparation process and became covalently incorporated into the hylan B. Analysis of the final radiolabeled hylan B showed that it was equivalent to that of hylan B produced during the standard manufacturing process and fulfilled all the specifications of hylan B.

Intradermal residence time of hylan B was assessed in the guinea pig model. The intradermal tissue compartment represents the proposed clinical route of administration for hylan B in the dermal tissue.

Blood elimination kinetics and tissue distribution of hylan B were studied in female Sprague Dawley rats following a single intravenous injection. The results from this study provide information about the systemic distribution and the route of elimination of hylan B.

#### **Intradermal Injection of [<sup>3</sup>H]-Hylan B ([<sup>3</sup>H]-Hylan gel) in Guinea Pigs (BXR 25407-F-I)**

This study was conducted to evaluate the intradermal residence time and local tissue reaction to hylan B. [<sup>3</sup>H]-hylan B was administered intradermally at 4 sites in 12 guinea pigs. The dose administered is estimated to be more than 50x the proposed clinical dose. At time points 0 (immediately following implantation), and 1, 2, and 4 weeks, skin injection tissue was harvested and analyzed for total radioactivity and histopathology. Recovery of radioactivity was used to estimate residence time in the tissue. Histopathological analysis was performed to verify the presence of hylan B in the tissue and to assess the local tissue reaction.

The results of this study indicate that after 4 weeks, approximately 90% of the injected radioactivity remains at the intradermal injection site. The presence of the hylan B implant was verified histologically at all time points. There was a minimal inflammatory–cell response in skin sections which appeared at week one and was present through week 4. The presence of this slight reaction may be related to the implantation procedure, which produces local physical separation of dermal collagen fibers. This effect has been observed in other similar studies in which hylan B was implanted intradermally into guinea pigs; long-term follow-up revealed that the tissue response diminishes and eventually resolves while the hylan B implant remains present in the tissue.

It is concluded that intradermal administration of [<sup>3</sup>H]-hylan B, at an amount that exceeds that estimated for clinical use, results in the formation of a stable biocompatible implant (90% present after 4 weeks) which is not associated with local or systemic toxicity in this animal model.

#### **Distribution of [<sup>3</sup>H]-Hylan Gel (degraded) in Rats (BXR 25212-F-I)**

This study was conducted to evaluate the kinetics and completeness of systemic elimination of hylan B. A large bolus of radiolabeled hylan B in degraded form was administered via intravenous injection in the rat. The gel particles were degraded by acid hydrolysis, which breaks the polymer backbone into smaller molecules while retaining the basic disaccharide repeating unit structure of the hyaluronan molecule. The dose administered was more than 100-fold higher than the proposed clinical dose and 1000-fold higher than the concentration of endogenous hyaluronan in rat blood.

Results from the study indicated that hylan B was eliminated quickly from the blood (> 75% by one hour) via urine, and very little radioactivity (< 2.5%) was recovered from vital body organs. Despite the extremely high mass of degraded hylan B injected intravenously, the half-life for blood elimination of the degraded material was rapid and similar to that for hyaluronan. There was no evidence of accumulation in any tissues involved in hyaluronan metabolism (liver, spleen, kidney). The major site of tissue uptake at each time point evaluated was found to be the liver. For all tissues showing any level of uptake, the radioactive content decreased regularly over the 24-hour time course. By the 24-hour time point, 90% of the radioactivity was detected in the urine and feces,

whereas only 2% of the injected radioactivity were detectable in the organs. Thus, degraded hylan B appears to follow the known blood elimination pathway for hyaluronan and does not exhibit hepatotoxic properties after direct intravenous injection of more than 100 times the proposed human clinical dose.

### **3.2.3 Pharmacodynamics**

#### **Six Month Study of Residence Time of [<sup>14</sup>C]-Hylan B Gel after Intradermal Administration in Female Guinea Pigs (BXR-25213-I)**

This study was conducted to evaluate the residence time, systematic distribution, and local tissue reaction to hylan B following intradermal administration. In this study, 18 female Hartley guinea pigs were injected intradermally with [<sup>14</sup>C]-hylan B into 8 sites on each animal. The total volume administered per animal corresponds to a dose more than fifty times the amount estimated for clinical use. Injection sites were harvested from study animals at time points 0 (immediately following implantation), 7 days, and 1, 3, and 6 months. Radioactivity was analyzed in 6 of 8 sites from each animal; the remaining two sites were evaluated for histopathology.

Dermal tissue analysis revealed substantial recovery of radioactivity at all time points through 6 months. A half-life time of 9.16 months was calculated from the data. At all time points, the appearance of the injection site skin was considered normal with no evidence of a treatment-related effect on the dermal tissue. There was no detectable inflammation or dermal fibrosis at any injection site. Analysis of major organs (lungs, liver, spleen, kidneys) from animals at the 3 and 6 month time points revealed no detectable radioactivity in these organs.

It is concluded that [<sup>14</sup>C]-hylan B is biologically compatible, is relatively stable in the dermal tissue, and does not produce any remarkable local tissue reaction or toxicity. There was no indication that all the test material was degraded or that it had migrated from the site of injection. Though a small decrease in recoverable radioactivity was observed over extended periods of time, there was no evidence that radioactivity was accumulating in major organs. Under the conditions of this study, [<sup>14</sup>C]-hylan B is stable and biologically compatible when injected into the dermal tissue of guinea pigs.

### **Intradermal and Subcutaneous Injection of Hylan Gel in Guinea Pigs (BXR 25408-F-I)**

This study was conducted to assess the behavior and long-term tissue effects of intradermally and subdermally injected hylan B as a potential soft tissue augmentation material. Sixteen Hartley guinea pigs were injected with hylan B, collagen (Zyplast®, Zyderm®) or saline control at twelve injection sites (six intradermal, six subdermal). Each implant site was palpated and examined for evidence of biocompatibility (erythema, edema, vesication, etc.). At 3 days, 1, 2, 4, 9, 13, 26, and 52 weeks, two animals were sacrificed and all injection sites were harvested, processed, and evaluated histologically.

Both collagen and hylan B produced an inflammatory response shortly after injection (by week two). This response was more prominent when either material was localized in the fat. With time, the inflammatory reaction diminished with hylan B and had essentially resolved by Week 26. The inflammatory response to collagen was more protracted.

Results indicate that hylan B is relatively stable and well tolerated when implanted into the dermal and subdermal tissue of guinea pigs. Hylan B was present at week 52 and was observed to be highly biocompatible with no inflammatory reaction associated with the hylan B implantation site.

#### **3.2.4 Basic Exploratory Studies**

These studies were conducted primarily to elucidate biological properties or to supplement existing experiments. While conducted in accordance with internal standards, they were not in strict compliance with GLP procedures. They do have scientific merit and confirm findings of existing studies and will be summarized here.

Intradermal injections in mice and subconjunctival implants in rabbits evaluated the irritation potential of hylan B in Basic Exploratory Studies **BXR 25302** and **25407-A**, respectively. No adverse tissue reactions were observed upon histological examination and microscopic evaluation revealed an absence of pathology or inflammation at the implantation site.

The immunological activity of hylan B was also assessed in six Basic Exploratory Studies. Four studies (**BXR 20004, 20005, 20006, 22222-F**) were conducted, whereby rabbits were injected submucosally (bladder) and or intramuscularly with hylan B, hylan

B with adjuvant (Freund's), hylan B and collagen, degraded hylan B, or hylan B-ovalbumin conjugate, and in some instances with an additional intradermal challenge of test material. The presence of serum antibody was determined by ELISA or by Passive Cutaneous Anaphylaxis (PCA) in female guinea pigs. Serum titers were observed to be very low and the guinea pig PCA did not detect any specific antibody production to hylan B in rabbits.

Hylan B immunogenicity was further evaluated in two additional studies. In study **BXR 22220-F**, owl monkeys having intravitreal hylan B implants received an intradermal injection of hylan B and were monitored for signs of erythema, edema, and irritation. Additionally, serum was tested for the presence of anti-hylan B antibodies using the guinea pig PCA. The intradermal injections were not associated with any edema, erythema, or signs of a hypersensitivity response and the guinea pig PCA did not detect any specific antibody production to hylan B.

In study **BXR 22227-F**, statistically significant complement activation did not result from exposure of normal human plasma to various concentrations of hylan B as analyzed by radioimmunoassay. The results from this study indicate that hylan B does not activate complement .

Cytotoxicity results obtained in GLP studies were substantiated by 6 Basic Exploratory Studies using different cell types: rabbit corneal endothelium (**BXR 26101**); mouse 3T3 fibroblasts (**BXR 22202-1 and -2**); bovine vascular endothelial cells (**BXR 22214**); and mouse L929 fibroblasts (**BXR 22223 and 22224**). Results indicate that hylan B, or extracts from hylan B, produced no evidence of toxicity in any of the test systems used.

A series of Basic Exploratory Studies confirmed that hylan B is not hemolytic, does not impair platelet function, and does not interfere with blood-clotting (**BXR 22203, 22205, 22216, 22217**).

Hylan B was tested in additional rabbit pyrogen studies according to USP (**BXR 25313**). None of the hylan B materials were pyrogenic.

Exploratory studies were conducted in primates to evaluate the potential reaction of living tissue to implanted hylan B. The owl monkey vitreous test was used as a sensitive quantitative method to evaluate the inflammatory (chemotactic) reaction in the vitreous and anterior chamber of the eye. Numerous batches of hylan B were tested and produced

no evidence of chemotactic reactions (**BXR 25310-A**). Hylan B was used as a viscosurgical device in glaucoma filtering surgery in owl monkeys by introducing hylan B gel under the conjunctiva to aid filtration of the aqueous. Hylan B was left in the anterior chamber for up to one year without any adverse reactions in the exposed tissues (**BXP 25087-F**). Hylan B was injected into the vitreous of owl monkey eyes. Electroretinography was performed at various times post-implantation. There was no significant difference between supramaximal ERG response of hylan B and control groups (**BXR 20202**).

The results of two Basic Exploratory Studies confirm the results from GLP studies that repeated injection of and/or long term exposure to hylan B produce no tissue reactions. Chronic effects (2-year) of hylan B were evaluated in rabbits (**BXR 25411-1**), by implanting material into the sphincter muscle and submucosa of the bladder. The histological evaluation indicated that no adverse histological reaction was elicited. The owl monkey eye test represents a long-term toxicity site where half of the liquid vitreous in the eye is replaced with hylan B. Batches of hylan B were implanted into the vitreous of owl monkeys (**BXR 25310-B** and **BXR 25310-C**). Post-implantation evaluation of the eyes determined that no sign of tissue pathology (retina, iris, cornea, anterior chamber) was noted.