

Long Term (90 Day) Muscle Implantation -

At least four pieces of 10 mm x 1 mm sections of [REDACTED] elastomer (designated as [REDACTED]) were loaded into a needle, steam sterilized, and then implanted by injection into rabbit muscle tissue as were at least 2 pieces of similarly sized negative control reference strips (USP plastic obtained from the US Pharmacopeial Convention). At 90 days, the 3 rabbits were euthanized and the paravertebral muscles dissected and fixed in neutral buffered formalin (NBF). Tissue sections were also excised and embedded in hematoxylin and eosin (H&E) for microscopic evaluation.

Mean macroscopic scores for test implant capsule formation or other signs of irritation were scored as follows:

- 0 - no capsule and no adverse reaction area
- 1 - up to 0.5 mm capsule or reaction area
- 2 - 0.6 to 1.0 mm capsule or reaction area
- 3 - 1.1 to 2.0 mm capsule or reaction area
- 4 - >2.0 mm capsule or reaction area

The test article [REDACTED] mean score minus the mean control score was 0.0. Under the conditions of this study, the macroscopic reaction of the test article was not significant as compared to the negative control material. Microscopically, the test article was classified as a nonirritant as compared to the reference control article.

USP Intracutaneous Toxicity Study -

A ratio of 60 cm²:20 ml (surface area of test article to volume of vehicle) was used for each preparation of [REDACTED] test article. The test article was extracted in 0.9 sodium chloride or cottonseed oil (CSO) for 1 hour at 121° C. Two rabbits were used per pair of extracts and 0.2 ml of test article or negative control blanks (SC or CSO extraction vehicles) were injected intracutaneously into 5 separate implant sites. Observations for erythema and edema were conducted at 24, 48, and 72 hours after injection and scored on a 0 to 4 basis. Under the conditions of this study, there was no evidence of significant irritation or toxicity from the [REDACTED] elastomer extracts injected intracutaneously into rabbits.

Ames Mutagenicity -

A Salmonella/mammalian microsome mutagenicity assay (Ames test) was conducted to determine whether a saline extract of 60 cm² of the [REDACTED] elastomer test article (designated by [REDACTED]) in

20 ml of saline for 1 hour, would cause mutagenic changes in histidine-dependent genetically altered strains (TA98, TA100, TA1535, TA1537, and TA1538) of Salmonella typhimurium.

Aliquots of the test extract, negative and positive control solutions were added to duplicate plates containing histidine-deficient medium. Separate plates were inoculated with Ames Salmonella tester strains. The rate of mutation to nonhistidine-dependent wild types was determined for each plate; the spontaneous reversion rate for each strain in the presence of the saline blank was compared to the corresponding rate of reversion in the presence of the test article extract and in the presence of known mutagens.

Under the conditions of this study, a saline extract of [REDACTED] elastomer did not cause mutagenic changes in the Salmonella typhimurium tester strains employed.

The elastomer [REDACTED] raw material, tested as cured slabs or extracts from the cured slabs, passed all of the above biocompatibility tests.

Elastomer [REDACTED] -

[REDACTED] has been biocompatibility tested as part of [REDACTED]. Because their testing used very similar sample preparation procedures, standardized test procedures, and the same testing laboratory Mentor would use for most of our raw materials testing, Mentor did not repeat the biocompatibility testing on this raw material. Instead, the reviewer is referred to [REDACTED] Group IV Biological Testing in the testing information, Sections 29 - 33 for the following reports:

- Cytotoxicity Study Using the ISO Agarose Overlay Method
- Kinetic-Chromogenic LAL Assay
- Cytotoxicity Study Using the ISO Elution Method
- Hemolysis Study - *IN VITRO* Procedure (Modified ASTM - Extraction Method)
- ISO Acute Systemic Toxicity in the Mouse (Saline, Alcohol, Polyethylene Glycol, Cottonseed Oil Extracts)
- ISO Acute Intracutaneous Reactivity Study in the Rabbit (Saline, Alcohol, Polyethylene Glycol, Cottonseed Oil Extracts)
- ISO Muscle Implantation Study in the Rabbit with Histopathology (One Week)
- ISO Muscle Implantation Study in the Rabbit with Histopathology (Twelve Weeks)
- Bacterial Reverse Mutation Assay (Saline and DMSO Extracts)
- Rabbit Pyrogen Study (Material Mediated)
- ISO Skin Irritation Study in the Rabbit (Single Exposure)

ISO Sensitization Study in the Guinea Pig (Maximization Method)(Saline and Cottonseed Oil Extracts)

ISO Sensitization Study in the Guinea Pig (Closed Patch Method)

The elastomer [REDACTED] raw material, tested as cured slabs or extracts from the cured slabs, passed all of the above biocompatibility tests.

[REDACTED] Silicone Gel [REDACTED] -

[REDACTED] silicone gel has been biocompatibility tested as part of [REDACTED]. Because their testing used very similar sample preparation procedures, standardized test procedures, and the same testing laboratory Mentor would use for most of our raw materials testing, Mentor did not repeat the biocompatibility testing on this raw material. Instead, the reviewer is referred to [REDACTED] Group IV Biological Testing in the testing information, Sections 15 to 20 for the following reports:

- Cytotoxicity Study Using the ISO Agarose Overlay Method
- LAL Kinetic-Chromogenic Assay
- Cytotoxicity Study Using the ISO Elution Method
- Hemolysis Study - *IN VITRO* Procedure (Extraction Method)
- ISO Acute Systemic Toxicity in the Mouse (Saline, Alcohol, Polyethylene Glycol, Cottonseed Oil Extracts)
- ISO Acute Intracutaneous Reactivity Study in the Rabbit (Saline, Alcohol, Polyethylene Glycol, Cottonseed Oil Extracts)
- ISO Muscle Implantation Study in the Rabbit with Histopathology (One Week)
- ISO Muscle Implantation Study in the Rabbit with Histopathology (Thirteen Weeks)
- Bacterial Reverse Mutation Assay (Saline and DMSO Extracts)
- Rabbit Pyrogen Study (Material Mediated)
- ISO Skin Irritation Study in the Rabbit (Single Exposure)
- ISO Sensitization Study in the Guinea Pig (Maximization Method)(Saline and Cottonseed Oil Extracts)

The [REDACTED] raw material, tested as cured gel, passed all of the above biocompatibility tests. [See Mentor's PMA #P910037/A49, dated April 9, 1999, Vol 7, pg 1311 for the [REDACTED]

Dispersion Coating [REDACTED]

Cured samples of Dispersion Coating [REDACTED] has been tested at various times in the past. [REDACTED] was tested in early 1994 at NAMSA with the following assays: Delayed Contact Sensitization, Ames Mutagenicity (Saline Extract), Ames Mutagenicity (DMSO extract). The material was cured and sterilized.

MEM Elution Cytotoxicity -

A cured sample of [REDACTED] Dispersion Coating and a laboratory supplied positive control were extracted in MEM at 37°C for 24 hours. MEM aliquots were used as the negative control. Microscopic scoring was based on monolayer confluence, vacuolization, swelling, crenation, and % lysis. Based upon the cytotoxic effect score and the controls performing as expected, the material was determined to be non-toxic.

Ames (DMSO extract) -

Testing was performed with the dimethyl sulfoxide extract of the test materials in the presence and absence of microsomal enzymes. The tester strains evaluated were: TA98, TA100, TA1535, TA1537, and TA1538. In comparison to the vehicle and positive controls, no positive mutagenic responses were observed with the test materials

Ames (Saline extract) -

Testing was performed with the saline extract of the test materials in the presence and absence of microsomal enzymes. The tester strains evaluated were: TA98, TA100, TA1535, TA1537, and TA1538. In comparison to the vehicle and positive controls, no positive mutagenic responses were observed with the test materials.

Dermal Sensitization -

Both saline and cottonseed oil extracts (60 cm² of test article to 20 ml vehicle extracted at 121°C for 1 hour) of the test materials were evaluated for delayed contact sensitization. Ten guinea pigs received intradermal injections of a 1:1 ratio mixture of the test material extract and Freund's Complete Adjuvant. After one week, the guinea pigs were exposed to filter paper patches saturated with the test material extract. Two weeks later, the animals were challenged with reapplication of patches saturated with the test material extract.

The challenge sites were scored over a 96 hour period for erythema and edema. Test material sites were compared to the negative control challenge (control vehicle) and the positive control (0.1% 1-chloro-2,4-dinitrobenzene (DNCB) solution). The saline and cottonseed oil test material extracts were not sensitizers.

[REDACTED] testing performed prior to 1994 is listed below. Those tests indicated (*) were performed at American Edwards Laboratories, except for the Rabbit

Intracutaneous Irritation which was performed at NAmSA. All results indicate that the material is non-toxic and biocompatible.

Agarose Overlay* -

Testing was performed in duplicate. Solid test samples of cured elastomer and positive and negative controls were placed upon a confluent cell monolayer. The positive controls had a score of 2 for the Zone Index and a score of 5 for the Lysis Index. The negative controls had a score of 0 for both the Zone Index and the Lysis Index. The test samples scored 1 for the Zone Index and 0 for the Lysis Index. Since the test and negative control scores were not significantly different, the material passed.

Mouse Acute Systemic Toxicity* -

A 60 cm² sample was extracted for 1 hour at 121°C in 20 ml normal saline and cottonseed oil. Mice were injected with either the test or control extracts of saline or cottonseed oil. The mice were observed at all time points (.5, 4, 24, 48, and 72 hours). Since no abnormal behavior was noted and no deaths occurred, the material passed.

Rabbit Intracutaneous Toxicity -

A 60 cm² sample of cured elastomer was extracted in 20 ml of sodium chloride and cottonseed oil for one hour at 121 °C. Two rabbits were injected with sodium chloride test extract at ten sites. Two other rabbits were injected with cottonseed oil test extract at ten sites. The sodium chloride or cottonseed oil extract controls (blank) were injected at five sites. All injection sites were examined at 24, 48, and 72 hours. Both erythema and edema were evaluated. The test and control sodium chloride injections sites all received a score of 0 (none) for erythema and edema. The erythema score at all cottonseed oil control and test sites was 1 (barely perceptible). The edema score at all cottonseed oil control and test sites received a score of 2 (well defined). Since the control and test sites did not differ, the material passed.

Short-term Intramuscular Implantation (30 days)* -

Two rabbits underwent intramuscular implantation for 30 days. Five test material samples and four negative controls were implanted. After sacrifice, gross evaluation was performed on the implantation sites. All test material implant sites were located and evaluated as negative for tissue damage. In one rabbit, one negative control was lost. All of the other negative control sites were located and evaluated as negative.

Long-term Intramuscular Implantation (90 days)* -

Three rabbits underwent a 90 day intramuscular implantation. Each animal received five test samples and four negative controls. One rabbit was euthanized after 6 weeks due to a cage accident that injured the hind foot. Samples were not retrieved from this animal. The remaining two rabbits were sacrificed as scheduled. All test and negative control implants were located. The sites were evaluated and scored as negative since tissue damage was not observed. Histopathology was also performed on the tissues. There was no evidence of toxicity and an insignificant variation between the tissue reactions to the test and negative control materials.

Blood Compatibility* -

The effect of the sample extract on hemolysis and blood clotting were determined. The percent hemolysis due to the presence of sample extract for the triplicate tubes ranged from 2.1 to 2.4%. Since this value was below 25%, the material passed. Blood clotting in the triplicate tubes containing the test sample extract occurred in 60 seconds. The acceptable range for clotting, based on +/- 25% of the negative control (60 seconds), was 45 to 75 seconds. Since the clotting time values were within this range, the material passed.

Xylene -

Xylene is used as the solvent in the [REDACTED] used to dip mammary shells. Because it's a solvent, xylene cannot be tested for biocompatibility by itself. Instead, gel-filled mammary shells containing the evaporated residues from the xylene used in the dispersions have been tested. The raw materials testing of [REDACTED] test the biocompatibility of the xylene residues. Finally, Mentor's other raw materials testing which employs the use of dipped Low Bleed shells ([REDACTED] mandrel raw materials testing in this section below) provides similar testing of xylene residues in device shell components.

PACKAGING FOR THE FINAL PRODUCT:**[REDACTED] Inner and Outer Thermoforms [REDACTED]**

Mentor uses inner and outer [REDACTED] thermoforms for [REDACTED] sterilization of gel-filled mammarys. The inner and outer thermoform parts, of which there are various sizes for each, are all made from the same materials. Testing has been performed on

both [REDACTED] sheeting from which the thermoforms are made and the thermoforms themselves. Copies of the laboratory test reports are located in the Biological Section Appendices. Testing [REDACTED] sheeting was performed in July of 1990 at NAMSA.

MEM Eluate -

A 56.4 cm² sample of [REDACTED] sheeting was extracted in 19 ml of MEM (minimum essential medium) at 37°C for 24 hours. A monolayer of L-929 mouse fibroblasts was grown to confluence and exposed to test, negative control (MEM), and positive control extracts. The cells were examined microscopically 24 hours after exposure. The positive control was toxic at a dilution of 1:2 at 24 hours. The test extract and the negative control were scored the same for the following parameters: confluent monolayer (+), intracellular granulation (-), swelling (-), crenation (-), percent lysis (0%), and CTE score N (nontoxic response). The material was evaluated as non-toxic under the conditions tested.

Agarose Overlay -

The test sample (1 cm² of solid test sample), negative control (USP plastic), and positive control (Latex) were placed upon a confluent cell monolayer. Following incubation for 24 hours, the L-929 mouse fibroblasts under the test and control materials were macroscopically and microscopically examined. The positive control was scored T (toxic) and Zone of Lysis 6 (mm). The test sample and negative control were score N (non-toxic) and Zone of Lysis 0 (mm). The test material was evaluated as non-toxic under the conditions tested.

Mouse Acute Systemic Toxicity -

A 60 cm² sample was extracted in 20 ml saline and cottonseed oil for 1 hour at 121°C. Mice were injected with either the test or control extract of the saline or cottonseed oil. Mice were observed for abnormal behavior at 0, 4, 24, 48, and 72 hours. All mice increased in weight. No mortalities or abnormal behaviors were observed. Since there was no significant difference between the test and control animals, the test article passed.

Rabbit Intracutaneous Irritation

A 60 cm² sample was extracted in 20 ml saline and cottonseed oil for 1 hour at 121°C. Two rabbits received five saline test extracts and five saline negative control injections. Another two rabbits received five cottonseed oil test extract and five cottonseed oil negative control injections. After 24, 48, and 72 hours, the injection sites were evaluated for erythema and edema. The test and control saline extract sites were scored 0 (none) for all time points. The test extract and control cottonseed oil extract sites were scored 1 (barely perceptible) at 24 and

48 hours. At 72 hours, the all sites were scored 0 (none). Since there was no significant difference between the control and test sites, the material passed.

The following test results are recent tests performed on polycarbonate thermoforms since 1999:

MEM Elution Method -

A 12 cm² sample from an extra large [REDACTED] sterilization inner thermoform was extracted in 5% Minimal Essential Medium (MEM) at 37⁰C for 24 hours. A MEM aliquot was used as the negative control and tin stabilized polyvinylchloride was used as a positive control. The test medium was incubated with L-929 mouse fibroblast cells for 24, 48, and 72 hours while the positive control was toxic at 24 hours. Both controls performed as expected. The test sample was found to be nontoxic.

Agarose Overlay Method

A 1 cm² sample from an extra large inner thermoform was used for testing. A 1.0 cm length of low density polyethylene was used as the negative control and a 1.0 cm x 1.0 cm piece of tin stabilized polyvinylchloride was used as the positive control. After a 24 hour incubation the culture was macroscopically examined and scored. The controls performed as expected. The test sample was determined to be nontoxic.

USP Intracutaneous Toxicity

36 cm² samples of an extra large inner thermoform were extracted in 12 ml of saline and cottonseed oil for 1 hour at 121⁰C. Extraction vehicles without test articles served as the control blanks. 0.2 ml doses of the test article extracts were injected intracutaneously into rabbits and observed for 24, 48, and 72 hours. Based upon the scoring results, there was no evidence of significant irritation or toxicity from the extracts of the test sample; therefore, the sample met the USP requirement.

USP Systemic Toxicity

36 cm² samples of an extra large inner thermoform were extracted in 12 ml of saline and cottonseed oil for 1 hour at 121⁰C. Extraction vehicles without test articles served as the control blanks. Mice were injected with either the test article extract or the corresponding control at a dose of 50ml/kg. The mice were observed for 4, 24, 48, and 72 hours. The results indicated that mouse body weights were acceptable, there was no mortality, and clinical observations were as expected or were clinically normal; therefore, the test article extracts met the USP requirement.

MEM Elution Method -

A 12 cm² sample from a small outer [REDACTED] sterilization thermoform was extracted in Minimal Essential Medium (MEM) at 37°C for 24 hours. A MEM aliquot was used as the negative control and tin stabilized polyvinylchloride was used as a positive control. The test medium was incubated with L-929 mouse fibroblast cells for 24, 48, and 72 hours while the positive control was toxic at 24 hours. Both controls performed as expected. The test sample was found to be nontoxic.

Agarose Overlay Method

A 1 cm² sample from a small outer thermoform was used for testing. A 1.0 cm length of low density polyethylene was used as the negative control and a 1.0 cm x 1.0 cm piece of tin stabilized polyvinylchloride was used as the positive control. After a 24 hour incubation the culture was macroscopically examined and scored. The controls performed as expected. The test sample was determined to be nontoxic.

MEM Elution Method -

A 12 cm² sample from a small inner [REDACTED] sterilization thermoform was extracted in Minimal Essential Medium (MEM) at 37°C for 24 hours. A MEM aliquot was used as the negative control and tin stabilized polyvinylchloride was used as a positive control. The test medium was incubated with L-929 mouse fibroblast cells for 24, 48, and 72 hours while the positive control was toxic at 24 hours. Both controls performed as expected. The test sample was found to be nontoxic.

Agarose Overlay Method

A 1 cm² sample from a small inner thermoform was used for testing. A 1.0 cm length of low density polyethylene was used as the negative control and a 1.0 cm x 1.0 cm piece of tin stabilized polyvinylchloride was used as the positive control. After a 24 hour incubation the culture was macroscopically examined and scored. The controls performed as expected. The test sample was determined to be nontoxic.

[REDACTED] Lid with [REDACTED] Adhesive [REDACTED]:

Mentor uses inner and outer [REDACTED] lids with [REDACTED] sterilization of gel-filled mammaries. The inner and outer lid parts, of which there are various sizes for each, are all made from the same materials and supplied by the same vendor to Mentor; therefore, testing of one size is indicative of the biocompatibility of all of these sizes and parts. Additional information relating to these lids can be found in

█ see the Biological Appendices for a █ access letter). Copies of Mentor's laboratory test reports are located in the Biological Section Appendices.

MEM Elution (Nelson Laboratories) -

A 60 cm² lid sample was extracted in MEM (Minimum Essential Medium) at 37 °C for 24 hours. Cells were grown to a confluent monolayer and exposed to test article, negative control, and positive control extracts as well as a reagent control (MEM). After 48 and 72 hours at 37°C the cells were microscopically examined. The negative and reagent controls were scored 0 for cytotoxicity. The sample and the negative control (MEM) extracts were scored 0 for cytotoxicity. The positive controls were scored 4. The sample extracts scored between 0 and 1 with most being 0. Since none of the test samples had a grade higher than 2, the material met the USP requirement.

ISO Elution Method -

A 24 cm² portion of lid sample was extracted in 4 ml Minimal Essential Medium (MEM) for 24 hours at 37°C. Low density polyethylene (15.1 cm² in 5 ml MEM) was similarly extracted as the negative control. MEM extracted without the test material was the reagent control. Tin stabilized polyvinyl chloride (25.4 cm² in 8.5 ml) was extracted in MEM as the positive control. The test medium and controls were incubated with L-929 mouse fibroblast cells for 48 hours. All controls performed as expected. The test sample extract was found to show no evidence of causing cell lysis or toxicity; therefore, it was determined to be not cytotoxic and met the requirements of the test.

Agarose Overlay -

A 1 cm² piece of test material, NAMSA negative, and tin stabilized polyvinyl chloride positive control were placed on a confluent cell monolayer. Following a 24 hour incubation, the cell were examined microscopically. The positive control had a score of 2 for the Zone of Lysis. The test material and the negative control had a score of 0. The material was determined to be nontoxic.

USP Systemic Toxicity -

A 72.0 cm² lid sample was extracted in 12 ml saline and cottonseed oil for one hour at 121 °C. Mice were injected with either the test or control extract of either saline or cottonseed oil. The mice were observed at 0, 4, 24, 48, and 72 hours after injection. The animal body weights were acceptable, no mortality occurred during the study, and no abnormal behavior occurred. For these reasons, the material showed no evidence of significant systemic toxicity from the extracts. The test article extracts met the USP requirements.

USP Intracutaneous Toxicity -

Using a ratio of 72.0 cm² test sample to 12 ml of saline and cottonseed oil, the lid sample was extracted for one hour at 121 °C. Two rabbits were injected at five sites with the test saline extract and at five sites with the control saline extract. Two other rabbits were injected similarly with the cottonseed oil test and control extracts. All sites were observed for erythema and edema at 24, 48, and 72 hours. All test and control saline injection sites were scored 0. Since the test and negative control sites were not significantly different, there was no evidence of significant irritation or toxicity. The test article extract met the USP requirements.

INDIRECT MANUFACTURING MATERIALS (contacts the device but is not a part of the finished device which is implanted):**Polyethylene Sheeting:**

This material is used as a temporary protective covering for elastomer sheeting and is removed during manufacturing. It is the temporary backing material for calendered, unvulcanized parts made from elastomer sheeting. The polyethylene material is not a component of the final product. Copies of the laboratory testing reports are located in the Biological Testing Appendices.

MEM Elution -

A 120 cm² sample of polyethylene sheeting was extracted in 20 ml of 5% Minimal Essential Medium (MEM) at 37°C for 24 hours. A MEM aliquot was used as the negative control and a vendor supplied positive control was used as a positive control. The test medium was incubated with L-929 mouse fibroblast cells for 24, 48, and 72 hours while the positive control was toxic at 24 hours. Both controls performed as expected. The test sample was found to be nontoxic.

Agarose Overlay, Solid -

A 1 cm² sample of polyethylene sheeting was used for testing. A 1.0 cm length of vendor supplied negative control was used as the negative control and a 0.5cm x 0.5cm piece of Latex was used as the positive control. After a 24 hour incubation the culture was macroscopically examined and scored. The controls performed as expected. The test sample was determined to be nontoxic.

USP Intracutaneous Toxicity -

Using a ratio of 120 cm² sample:20 ml extraction vehicle, polyethylene sheeting samples were extracted in saline and cottonseed oil for 72 hour at 50°C. Extraction vehicles without test articles served as the control blanks. 0.2 ml

doses of the test article extracts were injected intracutaneously into rabbits and observed for 24, 48, and 72 hours. Based upon the scoring results, there was no evidence of significant irritation or toxicity from the extracts; therefore, the test article met the USP requirement.

USP Systemic Toxicity -

Using a ratio of 120 cm² sample:20 ml extraction vehicle, polyethylene sheeting samples were extracted in saline and cottonseed oil for 72 hour at 50⁰C. Extraction vehicles without test articles served as the control blanks. Mice were injected with either the test article extract or the corresponding control at a dose of 50ml/kg. The mice were observed at 0, 4, 24, 48, and 72 hours. The results indicated that mouse body weights were acceptable, there was no mortality, and clinical observations were normal; therefore, the test article extracts met the USP requirement.

Mylar Sheeting:

Mylar sheeting comes in contact with silicone elastomer sheeting during processing. It is used as a temporary backing material during manufacturing for calendered vulcanized components made with elastomer sheeting. Copies of laboratory testing reports are located in the Biological Testing Appendices.

MEM Elution -

A 120 cm² sample of Mylar sheeting was extracted in 20 ml of 5% Minimal Essential Medium (MEM) at 37⁰C for 24 hours. A MEM aliquot was used as the negative control and a vendor supplied positive control was used as a positive control. The test medium was incubated with L-929 mouse fibroblast cells for 24, 48, and 72 hours while the positive control was toxic at 24 hours. Both controls performed as expected. The test sample was found to be nontoxic.

Agarose Overlay, Solid -

A 1 cm² sample of Mylar sheeting was used for testing. A 1.0 cm length of vendor supplied negative control was used as the negative control and a 0.5cm x 0.5cm piece of Latex #020 was used as the positive control. After a 24 hour incubation the culture was macroscopically examined and scored. The controls performed as expected. The test sample was determined to be nontoxic.

USP Intracutaneous Toxicity -

120 cm² Mylar sheeting samples were extracted in 20 ml saline and cottonseed oil for 1 hour at 121⁰C. Extraction vehicles without test articles served as the control blanks. 0.2 ml doses of the test article extracts were injected

intracutaneously into rabbits and observed for 24, 48, and 72 hours. Based upon the scoring results, there was no evidence of significant irritation or toxicity from the extracts; therefore, the test article met the USP requirement.

USP Systemic Toxicity -

Using a ratio of 120 cm² sample:20 ml extraction vehicle, Mylar sheeting samples were extracted in saline and cottonseed oil for 1 hour at 121°C. Extraction vehicles without test articles served as the control blanks. Mice were injected with either the test article extract or the corresponding control at a dose of 50ml/kg. The mice were observed at 0, 4, 24, 48, and 72 hours. The results indicated that mouse body weights were acceptable, there was no mortality, and clinical observations were normal; therefore, the test article extracts met the USP requirement.

Teflon Sheeting

Teflon sheeting is used to [REDACTED] when they are stored during the gel-filled mammary manufacturing process. The Teflon is removed during assembly of the device. Copies of the biological laboratory testing reports can be found in the Biological Section Appendices.

MEM Elution -

Teflon sheeting was extracted using a 120 cm² sample:20 ml of single strength Minimal Essential Medium (MEM) ratio at 37°C for 24 hours. A MEM aliquot was used as the negative control, and a vendor supplied positive control was used as a positive control. The test medium was incubated with L-929 mouse fibroblast cells for 24, 48, and 72 hours while the positive control was toxic at 24 hours. Both controls performed as expected. The test sample was found to be nontoxic.

Agarose Overlay, Solid -

A 1 cm² sample of Teflon sheeting was used for testing. A 1.0 cm length of USP negative control was used as the negative control and a 0.5cm x 0.5cm piece of Latex was used as the positive control. After a 24 hour incubation the culture was macroscopically examined and scored. The controls performed as expected. The test sample was determined to be nontoxic.

USP Intracutaneous Toxicity -

Using a ratio of 120 cm² sample:20 ml extraction vehicle, Teflon sheeting samples were extracted in saline and cottonseed oil for 1 hour at 121°C. Extraction vehicles without test articles served as the control blanks. 0.2 ml

doses of the test article extracts were injected intracutaneously into rabbits and observed for 24, 48, and 72 hours. Based upon the scoring results, there was no evidence of significant irritation or toxicity from the extracts; therefore, the test article met the USP requirement.

USP Systemic Toxicity -

Using a ratio of 120 cm² sample:20 ml extraction vehicle, Teflon sheeting samples were extracted in saline and cottonseed oil for 1 hour at 121⁰C. Extraction vehicles without test articles served as the control blanks. Mice were injected with either the test article extract or the corresponding control at a dose of 50ml/kg. The mice were observed for 4, 24, 48, and 72 hours. The results indicated that mouse body weights were acceptable, there was no mortality, and clinical observations were normal; therefore, the test article extracts met the USP requirement.

██████████ Mold Release:

██████████ Mold Release is a ██████████ mold release used in the calendering process when making elastomer sheeting. In order to perform the biocompatibility testing, a piece of ██████████ made using the mold release was used. Laboratory testing reports are located in the Biological Test Appendices.

MEM Elution -

Test material (60.2 cm²) was extracted under USP guidelines in 20 ml MEM (Minimum Essential Medium) for 24 hours at 37⁰C. Cells were exposed to the test sample, negative control (MEM), and positive control (vendor supplied) extracts. Sample and MEM control extracts were scored after 24, 48 and 72 hours as nontoxic. The positive control was scored as toxic at 24 hours. The material was determined to be nontoxic.

Agarose Overlay -

A 1 cm² test sample, a 1.0 cm length of vendor supplied negative control and a positive control (vendor supplied 0.5 x 0.5 cm piece of Latex) were placed upon a confluent cell monolayer. After a 24 hour incubation the positive control was scored as toxic. The negative control was determined to be nontoxic. The test material was determined to be nontoxic.

Hemolysis (Direct Contact) -

A total of 30.1 cm² of cut up sample was placed in 10 ml of 0.9% sodium chloride solution. Water and 0.9% sodium chloride without the test sample were used as negative and positive controls, respectively. To each sample was added

0.2 ml of rabbit blood collected in EDTA. The samples were then incubated at 37°C for 1 hour, at which time the samples were centrifuged. The absorbance value for the controls were used to calculate the percent hemolysis of the test article. The controls performed as expected, and the sample was determined to be non-hemolytic.

Hemolysis (Extraction Method) -

A 90.4 cm² test sample was placed in 30 ml of 0.9% sodium chloride solution and extracted at 37°C for 24 hours. Aliquots of 10 ml were used as the test solution. The positive control was 10 ml water and the negative control was 10 ml 0.9% sodium chloride solution. To these solutions was added 0.2 ml of rabbit blood. After mixing, the samples were incubated at 37°C for 1 hour, centrifuged, and then their spectrophotometric absorbance determined. The positive and negative controls performed as expected. The test sample was determined to be non-hemolytic.

USP Systemic Toxicity -

Using a ratio of 60 cm²:20 ml (surface area to extraction volume) [REDACTED] sheeting calendered using [REDACTED] Mold Release was extracted in 0.9% sodium chloride solution and cottonseed oil at 121°C for 1 hour. The extraction vehicles without the test article were used as the control blanks. Mice were injected with either the test article extract or the corresponding control at a dose of 50 ml/kg. The mice were observed at 0, 4, 24, 48, and 72 hours. The results indicated that mouse body weights were acceptable, there was no mortality, and all animals appeared clinically normal; therefore, the test extracts were not considered systemically toxic and met the USP requirements.

USP Intracutaneous Toxicity -

Using a ratio of 60 cm²: 20 ml (surface area to extraction volume) [REDACTED] sheeting calendered using [REDACTED] Mold Release was extracted in 0.9% sodium chloride solution and cottonseed oil at 121°C for 1 hour. The extraction vehicles without the test article were used as the control blanks. 0.2ml doses of the test article extracts were injected intracutaneously into rabbits and observed for 24, 48, and 72 hours. Based upon the scoring results, there was no evidence of significant irritation or toxicity from the extracts; therefore, the test article met the USP requirement.

Subcutaneous Implantation in the Rabbit (with Histopathology) (Four Weeks) -

[REDACTED] sheeting calendered with [REDACTED] Mold Release was used as the implant samples. A minimum of six 1 mm x 10 mm pieces were implanted subcutaneously into each rabbit. USP negative control strips (1 mm diameter) sterilized by steam were used as the control. At four weeks, the

subcutaneous tissue was examined and scored for capsule formation and other signs of irritation. Representative tissue samples were also excised for microscopic evaluation. All animals appeared clinically normal throughout the duration of the study. Weight gains of each rabbit were considered normal. Macroscopic scoring determined that there was no difference between the reactions of the test and control materials. Microscopic evaluation showed only a slight irritation compared to the USP negative control.

Subcutaneous Implantation in the Rabbit (with Histopathology) (90 Days) -

██████████ sheeting calendered with ██████████ Mold Release was used as the implant samples. A minimum of six 1 mm x 10 mm pieces were implanted subcutaneously into each rabbit. USP negative control strips (1 mm diameter) sterilized by steam were used as the control. At 90 days, the subcutaneous tissue was examined and scored for capsule formation and other signs of irritation. Representative tissue samples were also excised for microscopic evaluation. All animals appeared clinically normal throughout the duration of the study. Weight gains of each rabbit were considered normal. Macroscopic scoring determined that there was no difference between the reactions of the test and control materials. Microscopic evaluation showed only a slight irritation compared to the USP negative control.

Ames Mutagenicity Assay -

An Ames mutagenicity standard plate incorporation assay was conducted to determine whether a saline extract of ██████████ sheeting calendered with ██████████ Mold Release would cause mutagenic changes in histidine-dependent Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 in the presence and absence of S9 metabolic activation. A 60 cm² samples was extracted in 20 ml saline for 1 hour at 121°C. Saline without the test material was similarly processed for use as a negative control. The positive control used was Dexon (paradimethylaminobenzene diazosulfonic acid sodium salt). All Salmonella typhimurium strains exhibited appropriate genetic characteristics pertaining to this assay. No significant spot plate inhibition was seen. There was no two-fold or greater increase in the mean number of revertants of tester strains TA98, TA100, TA1535, TA1537, and TA1538 in the presence of a saline test article extract. The data obtained from this study met the criteria for a valid assay. For these reasons, the saline article extract was not considered to be mutagenic in this assay.

Systemic Antigenicity Study in the Guinea Pig -

Using a ratio of 60 cm²: 20 ml (surface area to extraction volume) ██████████ sheeting calendered using ██████████ Mold Release was extracted in 0.9% sodium chloride solution at 121°C for 1 hour. The extraction vehicles without the test article were used as the control blanks. The test article extract

was evaluated for antigenic potential following multiple injections in the guinea pig. The test article was injected intraperitoneally (IP) into six guinea pigs, three times a week (every other day) until six induction injections were conducted. Similarly, four additional guinea pigs were injected IP with 0.9% sodium chloride USP solution alone as the control condition. Thirteen days after the last IP injection, three of the six test guinea pigs were challenged by an intravenous injection (IV) of the test article and then observed for any signs of antigenicity. These signs included face pawing, ruffling of the fur, dyspnea, sneezing or coughing three times or more, retching, rales, prostration, convulsions, or death. Twenty-seven days later, the remaining three test animals and all of the control animals were challenged by an IV injection and observed for signs of antigenicity. There were no signs of antigenicity observed; therefore, the test article was not considered antigenic in the guinea pig.

Primary Skin Irritation Test in the Rabbit -

The test article used was a 1 inch x 1 inch piece of [REDACTED] sheeting calendered with [REDACTED] Mold Release. A test article was topically applied to the intact and abraded skin of six rabbits and left in place for 24 hours. The test sites were graded for erythema and edema at 24 and 72 hours after the single sample application. The scoring system used was the Draize scoring criteria recommended by the Federal Hazardous Substance Act. The primary irritation index of the test article was calculated to be 0.00. No irritation was observed on the skin of the rabbits. The test article was not considered to be a primary skin irritant.

[REDACTED]:

[REDACTED] is only used in the texturing process of Siltex gel-filled mammary prostheses. The [REDACTED] during the shell texturing process. It is then peeled off of the shell surface, and the shell cured. This imparts the roughened surface characteristics to the shell surface. Copies of laboratory testing reports on this material are located in the Biological Testing Appendices.

MEM Elution -

A 12.5 cm² sample of [REDACTED] was extracted in 4 ml of Minimum Essential Medium (MEM) for 24 hours at 37°C. A MEM aliquot was used as the negative control and a vendor supplied positive control was used as the positive control. The test medium was incubated with L-929 mouse fibroblast cells for 24, 48, and 72 hours while the positive control was toxic in 24 hours. Both controls performed as expected. The test sample was found to be nontoxic.

Agarose Overlay, Solid -

A 1 cm² sample of [REDACTED] was used for testing. A 1.0 cm length of vendor supplied negative control and 1.0 cm x 1.0 cm piece of vendor supplied positive control were used as the controls. After a 24 hour incubation in which the test and control samples were in direct contact with the agar overlay surface, the culture was macroscopically examined and scored. The controls performed as expected. The test sample was determined to be nontoxic.

USP Systemic Toxicity -

Using a ratio of 55.6 cm²:19 ml (surface area to extraction volume) [REDACTED] was extracted in 0.9% sodium chloride solution and cottonseed oil at 121°C for 1 hour. The extraction vehicles without the test article were used as the control blanks. Mice were injected with either the test article extract or the corresponding control at a dose of 50 ml/kg. The mice were observed at 0, 4, 24, 48, and 72 hours. The results indicated that mouse body weights were acceptable, there was no mortality, and all animals appeared clinically normal; therefore, the test extracts were not considered systemically toxic and met the USP requirements.

USP Intracutaneous Toxicity -

Using a ratio of 55.6 cm²:19 ml (surface area to extraction volume) [REDACTED] was extracted in 0.9% sodium chloride solution and cottonseed oil at 121°C for 1 hour. The extraction vehicles without the test article were used as the control blanks. 0.2 ml doses of the test article extracts were injected intracutaneously into rabbits and observed for 24, 48, and 72 hours. Based upon the scoring results, there was no evidence of significant irritation or toxicity from the extracts; therefore, the test article met the USP requirement.

[REDACTED] Mandrels:

[REDACTED] mandrels are used in the dip molding of high temperature vulcanizing silicone mammary shells. The mandrel shape and size determine the shape and size of the shell formed. [REDACTED] is used primarily due to its chemical resistance to the solvents used in the dipping process and heat resistance to the high heat used during the silicone curing process. The mandrel material never contacts the patient only the device during shell dipping. Copies of laboratory testing reports on this material from 2000 are summarized below and are located in the Biological Testing Appendices.

USP Systemic Toxicity -

The test article used in this testing was a gel-filled mammary shell dipped on [REDACTED] mandrel. A 32.5 cm² piece of shell was extracted in 11 ml (surface

area to extraction volume) of 0.9% sodium chloride solution, cottonseed oil, alcohol in saline (1:20), and polyethylene glycol 400 (PEG) at 121°C for 1 hour. The extraction vehicles without the test article were used as the control blanks. Mice were injected with either the test article extract or the corresponding control at a dose of 50 ml/kg (10 g/kg for PEG). The mice were observed at 0, 4, 24, 48, and 72 hours. The results indicated that mouse body weights were acceptable and there was no mortality. The animals appeared clinically normal except for the test and control mice injected with alcohol in saline and cottonseed oil. The former appeared lethargic immediately after injection while the latter appeared ungroomed 4 hours after dosing. The alcohol injected animals had an expected pharmacological reaction due to the alcohol content of the extract while the cottonseed oil injected animals observation was considered an expected effect due to the unctuous nature of the extract. The test extracts were not considered systemically toxic and met the USP requirements.

USP Intracutaneous Toxicity -

A 32.5 cm² piece of shell was extracted in 11 ml (surface area to extraction volume) of 0.9% sodium chloride solution, cottonseed oil, alcohol in saline (1:20), and polyethylene glycol 400 (PEG) at 121°C for 1 hour. The extraction vehicles without the test article were used as the control blanks. 0.2 ml doses of the test article extracts were injected intracutaneously into rabbits and observed for 24, 48, and 72 hours. Based upon the scoring results, there was no evidence of significant irritation or toxicity from the extracts; therefore, the test article met the USP requirement.

USP Muscle Implantation in the Rabbit (One Week) -

Pieces of high temperature vulcanized silicone mammary shell dipped using [REDACTED] mandrel were used for implantation. A minimum of six sections of the test article (1mm x 10 mm) per rabbit were placed into the muscle of two rabbits. USP negative control strips were used for the controls. At one week, the rabbits were weighed and capsule formation or other signs of irritation in the muscle were scored. Clinically, all animals appeared normal with acceptable body weight data. There was no visible reaction at any test or control implant site; therefore, there was not significant tissue contact irritation.

Testing performed prior to 1990 on [REDACTED] is summarized below. Copies of laboratory testing reports are located in the Biological Testing Appendices.

Agar Overlay -

A 1 cm² sample of [REDACTED] was used for testing. A piece of USP negative control plastic and a piece of latex as the positive control were used as the controls. After a 24 hour incubation in which the test and control samples

were in direct contact with the agar overlay surface, the culture was macroscopically examined and scored. The controls performed as expected. The test sample was determined to be nontoxic.

MEM Elution -

67.7 cm² sample of [REDACTED] was extracted in 22.6 ml Minimal Essential Medium (MEM) for 24 hours at 37°C. An MEM aliquot served as the negative control. The extract was incubated with the cells for up to 72 hours. Based on the scoring system used the [REDACTED] extract was determined to have an intermediate response at 48 and 72 hours. The controls performed as expected.

Acute Systemic Toxicity

6 gm of [REDACTED] were extracted in 30 ml cottonseed oil at 121°C for 1 hour. When injected into mice and the reaction compared to mice injected with the blank, the extract passed the test because there was no significantly greater reaction in the treatment animals than in the blank animals.

Intracutaneous Toxicity

6 gm of [REDACTED] were extracted in 30 ml cottonseed oil at 121°C for 1 hour. 0.2 ml of the extract and the extract vehicle blank were injected intracutaneously into rabbits and examined at 24, 48, and 72 hours after injection. Based on the scoring system, the extracted material met the test requirements because there was no significant difference between the extract and blank results.

[REDACTED] Mandrels:

[REDACTED] mandrels are an alternative mandrel for dip molding of high temperature vulcanizing silicone mammary shells. The mandrel shape and size determine the shape and size of the shell formed. [REDACTED] mandrels are used primarily due to their chemical resistance to the solvents used in the dipping process, heat resistance to the high heat used during the silicone curing process, and expected longevity. The mandrel materials never contact the patient only the device during shell dipping. Besides some testing of shells dipped on [REDACTED] mandrels, the [REDACTED] has been biologically tested as part of prototype [REDACTED] ceramic mandrels from which shells were dipped and submitted for biological testing. Finally, silicone gel-filled mammary shells in contact with [REDACTED] were tested to qualify [REDACTED]. Copies of laboratory testing reports on this material are located in the Biological Testing Appendices.

██████████ Mandrel Shell Testing

MEM Elution Method -

A 24 cm² piece of shell was extracted with 4 ml Minimal Essential medium (MEM) at 37°C for 24 hours. Aliquots of MEM without test material but subjected to the extraction conditions were used as the negative control. Tin stabilized polyvinyl chloride was used as the positive control (25.4 cm² extracted in 8.5 ml of MEM). The test medium and controls were incubated with L-929 mouse fibroblast cells for up to 72 hours. All controls performed as expected. The test sample extract was not considered toxic under the conditions of this study.

Agarose Overlay Method (Solid) -

A 1 cm² sample of shell was used for testing. A 1.0 cm length of vendor supplied high density polyethylene was used as the negative control. A 1.0 cm x 1.0 cm piece of tin stabilized polyvinyl chloride was used as the positive control. After a 24 hour incubation in which the test and control samples were in direct contact with the agar overlay surface, the culture was macroscopically examined and scored. The controls performed as expected. The test sample showed no evidence of causing cell lysis or toxicity. The test article was not considered toxic.

██████████ Qualification

ISO Elution Method -

A 12 cm² sample of shell dipped on ██████████ ceramic mandrel was extracted in 4 ml of Minimum Essential Medium (MEM) for 24 - 26 hours at 37°C. Aliquots of MEM without test materials but subjected to the extraction conditions were used as the reagent controls. A vendor supplied low density polyethylene material was extracted in MEM as the negative control (15.1 cm² extracted in 5 ml MEM). Tin stabilized polyvinyl chloride was used as the positive control (25.4 cm² extracted in 8.5 ml of MEM). The test medium and controls were incubated with L-929 mouse fibroblast cells for 48 hours. All controls performed as expected. The test sample extract was found to show no evidence of causing cell lysis or toxicity; therefore, it was determined to be not cytotoxic and met the requirements of the test.

Agarose Overlay Method -

A 1 cm² sample of shell dipped on ██████████ ceramic mandrel was used for testing. A 1.0 cm length of vendor supplied low density polyethylene was used as the negative control. A 1.0 cm x 1.0 cm piece

of tin stabilized polyvinyl chloride was used as the positive control. After a 24 – 26 hour incubation in which the test and control samples were in direct contact with the agar overlay surface, the culture was macroscopically examined and scored. The controls performed as expected. The test sample showed no evidence of causing cell lysis or toxicity. The test article met the requirements of the test.

ISO Acute Systemic Toxicity -

A 43 cm² sample of shell dipped on [REDACTED] ceramic mandrel was extracted in 14 ml of 0.9% sodium chloride solution and cottonseed oil, NF for 1 hour at 121⁰C. Aliquots of the extract vehicles without test articles were used as the reagent controls. Mice were injected with either the test article extract or the corresponding control at a dose of 50 ml/kg. The saline was injected intravenously while the cottonseed oil was injected intraperitoneally. The mice were observed at 0, 4, 24, 48, and 72 hours. The results indicated that mouse body weights were acceptable and there was no mortality. All animals appeared clinically normal except that the test and control animals injected with cottonseed oil, NF appeared ungroomed 4 hours after dosing. This observation was an expected effect due to the unctuous nature of the extract. For these reasons, the test extracts were not considered systemically toxic and met the USP requirements.

ISO Acute Intracutaneous Reactivity -

A 43 cm² sample of shell dipped on [REDACTED] ceramic mandrel was extracted in 14 ml of 0.9% sodium chloride solution and cottonseed oil, NF for 1 hour at 121⁰C. Aliquots of the extract vehicles without test articles were used as the reagent controls. 0.2 ml doses of the test article extracts or controls were injected intracutaneously into rabbits and observed for 24, 48, and 72 hours. Based upon the scoring results, there was no evidence of irritation or toxicity from the extracts. The Primary Irritation Index Characterization for the extracts was negligible.

[REDACTED] Qualification

MEM Elution -

A 12 cm² piece of shell in contact [REDACTED] was extracted in 4 ml of Minimum Essential Medium (MEM) at 37⁰C for 24 hours. . A MEM aliquot was used as the negative control and a vendor supplied positive control was used as the positive control. The test medium was incubated with L-929 mouse fibroblast cells for 24, 48, and 72 hours

while the positive control was toxic in 24 hours. Both controls performed as expected. The test sample was found to be nontoxic.

Agar Overlay -

A 1 cm² sample of shell in contact with [REDACTED] was used as the test article. An aliquot of 0.1 ml of the test article was applied onto a filter disc. A filter disc control with 0.1 ml of 0.9% sodium chloride irrigation USP, a 1.0 cm piece of vendor supplied negative control plastic, and 1.0 cm x 1.0 cm piece of vendor supplied positive control were used as the controls. After a 24 hour incubation in which the filter discs and control samples were in direct contact with the agar overlay surface, the cultures were macroscopically examined and scored. The controls performed as expected. The test sample was determined to be nontoxic.

USP Systemic Toxicity -

The test article used in this testing was a shell in contact with [REDACTED]. Using a ratio of 60 cm²: 20 ml extract vehicle (surface area to extraction volume), a sample was extracted in 0.9% sodium chloride solution and cottonseed oil, NF at 121°C for 1 hour. The extraction vehicles without the test article were used as the control blanks. Mice were injected with either the test article extract or the corresponding control at a dose of 50 ml/kg. The mice were at 0, 4, 24, 48, and 72 hours. The results indicated that mouse body weights were acceptable, there was no mortality, and the animals appeared clinically normal throughout the study. The test extracts were not considered systemically toxic and met the USP requirements.

USP Intracutaneous Toxicity -

The test article used in this testing was a shell in contact with [REDACTED]. Using a ratio of 60 cm²: 20 ml extract vehicle (surface area to extraction volume), a sample was extracted in 0.9% sodium chloride solution and cottonseed oil, NF at 121°C for 1 hour. The extraction vehicles without the test article were used as the control blanks. 0.2 ml doses of the test article extracts and control were injected intracutaneously into rabbits and observed for 24, 48, and 72 hours. Based upon the scoring results, there was no evidence of significant irritation or toxicity from the extracts; therefore, the test article met the USP requirement.

Polyethylene Bags

Polyethylene bags are used in multiple processes during the fabrication of gel-filled mammary prostheses. Because the bags are sourced from a single vendor and are made from the same raw material, testing one size of polyethylene bag provides data indicative of all polyethylene bags used in this manufacturing process.

ISO Elution Method -

A 72 cm² portion of the test material was extracted with 12 ml Minimal Essential Medium (MEM) at 37°C for 24 hours. High density polyethylene was extracted as the negative control (30 cm² extracted with 10 ml), tin stabilized polyvinylchloride was extracted as the positive control (60.1 cm² extracted with 20 ml), and an aliquot of MEM extracted without the test material served as the reagent control. Test extracts and the controls were incubated with fibroblast cells for 48 hours. The test extracts scored 0 while the controls performed as expected; therefore, the test extract met the requirements of the test.

USP and ISO Systemic Toxicity -

A 72 cm² portion of the test material was extracted with 12 ml 0.9% sodium chloride solution USP (SC) or cottonseed oil, NF (CSO) at 70°C for 24 hours. The extraction vehicle without the test article served as the control blank. The SC extract was injected intravenously while the CSO extract was injected intraperitoneally. The animals were observed at 0, 4, 24, 48, and 72 hours after injection. The animals were weighed at the 72 hour observation. Body weights were acceptable, there was no mortality during the study. All animals appeared clinically normal except that the test and control animals injected with cottonseed oil, NF appeared ungroomed 4 hours after dosing. This observation was an expected effect due to the unctuous nature of the extract. For these reasons, the test extracts were not considered systemically toxic and met the test requirements.

ISO Intracutaneous Study -

A 72 cm² portion of the test material was extracted with 12 ml 0.9% sodium chloride solution USP (SC) or cottonseed oil, NF (CSO) at 70°C for 24 hours. The extraction vehicle without the test article served as the control blank. 0.2 ml doses of the test article extracts and control were injected intracutaneously into rabbits and observed for 24, 48, and 72 hours. The Primary Irritation Index Characterization for the extracts was negligible. There was no evidence of irritation or toxicity from the extracts.

Isopropyl Alcohol:

Isopropyl alcohol is used to wash silicone components, including the shell, during several steps in the manufacturing process. It is also used to wipe down working areas where the device and components are assembled. The solvent contacts the device but is evaporated during the manufacturing steps involving elevated temperatures over a period of time. For that reason, the alcohol from the manufacturing process does not contact the patient.

Each incoming lot of this material is tested to ensure that it is spore free before use in production. Vendor certification states that the alcohol is electronic/semi-conductor grade to minimize any chance of residuals.

BIOLOGICAL TESTING OF FINISHED DEVICES OR COMPONENTS FROM FINISHED DEVICES

The testing described in the previous section indicates that the raw materials used in the Smooth and Siltex Gel-filled Mammary Prostheses are non-toxic, non-immunogenic, and biocompatible. Finished component and/or finished device testing have also been performed to demonstrate that the manufacturing processes to make components and assemble, package, and sterilize the final device do not create a device with toxic or non-biocompatible properties. These tests cover pyrogenicity testing through more complex testing related to mutagenicity, chronic toxicity/carcinogenicity, immunogenicity, autoimmunity, and reproduction/teratogenicity.

Mentor manufactures smooth and textured styles of gel-filled mammary prostheses in an array of sizes and shapes. In many cases, the same materials are used for the same components in different device styles and product lines. Similar processing conditions are used to assemble the different final devices from these components. In order to ensure that **finished product** biological testing encompasses all components and materials found in all the styles of gel-filled mammary prostheses, Mentor has conducted testing on a selected set of finished devices (and components in some cases) chosen to represent the full range of components and materials. Therefore, not all final configurations and sizes of these devices have undergone separate biological testing. For example, Siltex textured gel-filled device shells are used for the Siltex Moderate Profile, Siltex Moderate Plus, and Siltex High Profile Gel-filled Prostheses. Because the shells for the three device lines are made from the same raw materials using the same manufacturing processes (except for the shape of the mandrel), testing one type of shell is sufficient to cover all product lines. Finally, all testing in this section was performed on shells dipped with [REDACTED] mandrels. Repeating these tests for shells dipped [REDACTED] mandrels was determined to be not necessary since chemical extractables testing on finished devices showed the extractables profile of devices dipped on both mandrel materials to be equivalent (data to be provided in the Chemical Testing Section of this PMA).

Biological testing conducted on finished products included the following:

1. Cytotoxicity (ISO Agarose Overlay & ISO Elution)
2. ISO Acute Intracutaneous Reactivity (Rabbit)
3. ISO Acute Systemic Toxicity (Mouse)
4. Hemolysis (Direct Contact & Extraction)
5. Material Mediated Pyrogenicity
6. Mutagenicity
 - A. Bacterial Reverse Mutation Assay - saline and ethanol extracts
 - B. Unscheduled DNA Synthesis Assay in Mammalian Cells In Vitro - saline and ethanol extracts
 - C. Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells - saline and ethanol extracts
7. Immunotoxicity

8. Autoantibody Production
9. Adjuvancy
10. Reproduction/Teratology
11. Chronic Toxicity/Carcinogenicity
12. ISO Sensitization (Maximization Method)

The above tests were performed on the following device/component test samples:

Device/Component Tested	Biological Tests Performed
Smooth Gel-filled Prosthesis:	
Sterile Device ¹	1, 2, 3, 4, 5, 6
Smooth Shell Only ²	7, 8, 10, 11
Siltex Gel-filled Prosthesis:	
Sterile Device ¹	1, 2, 3, 4, 5
Textured Shell Only ²	7, 9 ³ , 10, 11
Gel Only ⁴ :	7, 9, 10, 11
Laser Marked Patches:	1, 2, 3, 12

- ¹ - Made with [REDACTED]
- ² - Made with [REDACTED] shell dispersions (an equivalent material from a former vendor)
- ³ [REDACTED] textured layer on RTV shell
- ⁴ - [REDACTED]

STERILE GEL-FILLED DEVICE TESTING - [REDACTED]

Mentor Siltex and Smooth Low Bleed Gel-filled Mammary Prostheses were tested in 1999 for biological compatibility (see HS33.990226.02 "Biological Testing Results of [REDACTED] Devices Vendor Qualification" in the Appendices of this section) as part of the qualification of a new silicone material vendor [REDACTED] and [REDACTED].

Acute biological tests were performed on sterile finished devices made as part of the product performance qualification for these device changes. Longer term biological tests were not repeated because the replacement materials were made using processes and raw materials very similar to the replaced silicone materials, [REDACTED] testing indicated the replacement materials were very similar to the replaced materials, and the finished device chemical extractable profile of the new vendor devices were substantially equivalent to the replaced devices.

The biological qualification results for these gel-filled finished devices made with [REDACTED] materials are summarized below.

ISO ELUTION METHOD (see HS33.990226.02) -

Siltex Gel-filled Prosthesis, Lot #188747: The entire device was extracted in single strength Minimum Essential Medium (161.1 cm² surface area in 54 ml

of extraction vehicle) at 37°C for 24 - 26 hours. Aliquots of MEM without the test material but subjected to the extraction conditions were used as the reagent controls. A vendor supplied low density polyethylene material was extracted in MEM as the negative control (15.1 cm² extracted in 5 ml MEM). Tin stabilized polyvinyl chloride was used as the positive control (25.4 cm² extracted in 8.5 ml of MEM). The test medium and controls were incubated with L-929 mouse fibroblast cells for 48 hours. All controls performed as expected. The test article extracts showed no evidence of causing cell lysis or toxicity; therefore, it met the requirement of the tests.

Smooth Gel-filled Prosthesis, Lot #188759: This device was tested in an identical manner to the Siltex Gel-filled Prosthesis described above with an identical outcome.

ISO AGAROSE OVERLAY (see HS33.990226.02) -

Siltex Gel-filled Prosthesis, Lot #188747: The entire device was extracted in 0.9% sodium chloride irrigation, USP (161.1 cm² surface area in 54 ml of extraction vehicle). A 0.1 ml aliquot of the extract was applied to a paper filter disc and placed on the agarose surface. A 1.0 cm length of vendor supplied low density polyethylene negative control and a vendor supplied 1 cm² piece of tin stabilized polyvinyl chloride positive control were placed upon a confluent cell monolayer. The control samples performed as anticipated. The test article extract showed no evidence of causing cell lysis or toxicity; therefore, it met the requirements of the test.

Smooth Gel-filled Prosthesis, Lot #188759: This device was tested in an identical manner to the Siltex Gel-filled Prosthesis described above with an identical outcome.

ISO ACUTE SYSTEMIC TOXICITY (see HS33.990226.02) -

Siltex Gel-filled Prosthesis, Lot #188747: The entire device was extracted in 0.9% sodium chloride irrigation, USP (SC) and cottonseed oil, NF (CSO) (483.3 cm² surface area in 161 ml SC and 161.1 cm² surface area in 54 ml of CSO) for 1 hour at 121°C. Mice were injected with a test extract or control. SC was injected intravenously and CSO was injected intraperitoneally. The mice were observed at 0, 4, 24, 48, and 72 hours. The results indicated that mouse body weights were acceptable and there was no mortality. The animals appeared clinically normal except that the test and control mice injected with cottonseed oil appeared ungroomed 4 hours after dosing. This observation was considered an expected effect due to the unctuous nature of the extract. The test extracts were not considered systemically toxic and met the test requirements.

Smooth Gel-filled Prosthesis, Lot #188759: This device was tested in an identical manner to the Siltex Gel-filled Prosthesis described above with the same outcome.

ISO ACUTE INTRACUTANEOUS REACTIVITY (see HS33.990226.02) -

Siltex Gel-filled Prosthesis, Lot #188747: The entire device was extracted in 0.9% sodium chloride USP solution and cottonseed oil, NF (483.3 cm² surface area was extracted in 161 ml SC and 161.1 cm² was extracted in 54 ml CSO at 121⁰C for 1 hour). The extraction vehicles without the test article were used as the control blanks. 0.2 ml doses of the test article extracts were injected intracutaneously into rabbits and observed for 24, 48, and 72 hours. Based upon the scoring results, there was no evidence of irritation or toxicity from the extracts. The Primary Irritation Index Characterization for the extracts was negligible.

Smooth Gel-filled Prosthesis, Lot #188759: This device was tested in an identical manner to the Siltex Gel-filled Prosthesis described above with the same outcome.

HEMOLYSIS (DIRECT CONTACT) (see HS33.990226.02) -

Siltex Gel-filled Prosthesis, Lot #188747: The entire device was covered with 0.9% sodium chloride USP solution (SC) (161.1 cm² surface area to 54 ml SC). Water was used as the positive control while 24.1 cm² low density polyethylene covered with 8 ml SC was used as the negative control. To each sample was added rabbit blood collected in EDTA in the ratio of 1 ml diluted blood:8ml vehicle. The samples were then incubated in a stationary position at 37⁰C for 4 hour, at which time the samples were centrifuged per the standard procedure. The absorbance value of the positive control was used to calculate the hemolytic index of the test article. The controls performed as expected, and the test sample's hemolytic index was determined to be 0.0%. The test article was non-hemolytic.

Smooth Gel-filled Prosthesis, Lot #188759: This device was tested in an identical manner to the Siltex Gel-filled Prosthesis described above with an identical outcome.

HEMOLYSIS (EXTRACTION METHOD) (see HS33.990226.02) -

Siltex Gel-filled Prosthesis, Lot #188747: The entire device was extracted with 0.9% sodium chloride USP solution (SC) (161.1 cm² surface area to 54 ml SC) for 1 hour at 121⁰C. Ten ml water processed using the same extraction conditions as the test article served as the positive control while 60.4 cm² low density polyethylene extracted in 20 ml SC similar to the test article served as the negative control. A 1.0 ml aliquot of diluted blood was added to 8.0 ml test

extract or control vehicle. Following the standard testing procedure, the test article hemolytic index was determined. The test sample's hemolytic index was determined to be 0.0%, and the controls performed as expected. The test article extract was non-hemolytic.

Smooth Gel-filled Prosthesis, Lot #188759: This device was tested in an identical manner to the Siltex Gel-filled Prosthesis described above with an identical outcome.

MATERIAL MEDIATED PYROGENICITY (see HS33.990226.02) -

Siltex Gel-filled Prosthesis, Lot #188747: The entire device was extracted in 0.9% sodium chloride USP solution (483.3 cm² surface area to 161 ml extraction vehicle) for 1 hour at 121°C. No more than 30 minutes prior to injection, the temperature of three New Zealand White Rabbits were recorded. The rabbits then receive an intravenous injection of the extract at a dose of 10 ml/kg body weight. Rabbit temperatures were recorded at 30 minute intervals between 1 and 3 hours after injection. No single animal showed a temperature rise of 0.5°C above its base line during the 3 hour observation period; therefore, the extract was judged nonpyrogenic.

Smooth Gel-filled Prosthesis, Lot #188759: This device was tested in an identical manner to the Siltex Gel-filled Prosthesis described above with the same outcome.

MUTAGENICITY TESTING

(Note - Mentor's Siltex Becker Mammary Prosthesis, which uses the same textured shell as the Siltex Gel-filled Low Bleed Mammary Prostheses, has also been tested for mutagenicity and was found to pass the same tests as the smooth shell described below.)

Bacterial Reverse Mutation Assay Conducted With Test Article Extracts

A sterile, finished device Smooth Low Bleed Gel-filled Mammary Prosthesis (275cc, [REDACTED] elastomer, [REDACTED]) was evaluated for its mutagenic potential by measuring the ability of its extracts to induce reverse mutations at selected loci of several strains of *Salmonella typhimurium* and at the tryptophan locus of *Escherichia coli* WP2 *uvrA* in the presence and absence of S9 activation. This testing was performed by BioReliance as a GLP study.

Test Article Preparation: The test articles were extracted in saline and ethanol following USP extract ratio requirements. The extractions were based on a ratio of 0.2 g of test article per 1 mL of extraction medium, saline or ethanol.

The test article was cut into small pieces, through all layers, placed in an extraction vessel and immersed in the required volume of extraction medium. The headspace of the vessel was purged with nitrogen gas. Each test article extract was incubated for 72 ± 2 hours at $50 \pm 2^{\circ}\text{C}$ with shaking. Saline and ethanol extraction blanks were prepared in the same manner, without the addition of test article. All extracts and extraction blanks were used within 24 hours of preparation.

Test System: The tester strains (TA98, TA100, TA1535, and TA1537) used in this assay were received from Dr. Bruce Ames. The strains have specific mutations in the histidine operon which block the histidine biosynthetic pathway resulting in histidine dependence (auxotrophy). Subsequent mutations allow the strains to revert and regain histidine independence (prototrophy). The *E. coli* tester strain was received from the National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland.

Tester strain TA98 and TA1537 detect frameshift mutations by reversion from histidine dependence to histidine independence. Tester strains TA1535 and TA100 detect base pair substitutions, primarily at GC residues, in the his G46 gene. Tester strain TA100 also detects frameshift mutations. Specificity of the reversion mechanism in *E. coli* is sensitive to base-pair substitution mutations.

Metabolic Activation: The metabolic activation system used in this assay was the Aroclor 1254-induced rat liver S-9 microsomal enzyme fraction. The S-9 was prepared from male Sprague-Dawley rats induced with a single 500 mg/kg intraperitoneal injection of Aroclor 1254 five days before sacrifice. After sacrifice, the liver was excised and homogenized. The S-9 microsomal enzyme fraction was prepared, aliquoted, and stored at approximately -70°C . The microsomal enzyme mixture (S-9 mix) was prepared immediately before its use in the mutagenesis assay.

Tester Strain Controls: All tester strain cultures were evaluated for the correct genotype and characteristic mean number of spontaneous revertants in the presence of the vehicle control solutions. The presence of the *rfa* deep rough mutation and deletion in the *uvrB* gene were confirmed in all *Salmonella* tester strain cultures. The presence of the pKM101 plasmid was confirmed in TA98 and TA100 cultures. All WP2 *uvrA* cultures demonstrated the deletion of the *uvrA* gene.

Negative Controls: Vehicle controls consisted of 0.9% saline and 100% ethanol and were added to cell cultures at the same concentration as the highest test article extract concentration.

Positive Controls: Since the tester strains used in the assay respond to different classes of chemical mutagens, the different strains were challenged with an appropriate agent to demonstrate an acceptable increased mutation response.

The positive controls for the various tester strains and assay conditions are provided below:

<u>Strain</u>	<u>With S-9</u>	<u>Positive Controls</u>	<u>Conc./Culture Plate</u>
TA98	Yes	2-aminoanthracene	1.0 ug
TA98	No	2-nitrofluorene	1.0 ug
TA100	Yes	2-aminoanthracene	1.0 ug
TA100	No	Sodium azide	1.0 ug
TA1535	Yes	2-aminoanthracene	1.0 ug
TA1535	No	Sodium azide	1.0 ug
TA1537	Yes	2-aminoanthracene	1.0 ug
TA1537	No	9-aminoacridine	75 ug
WP2 <i>uvrA</i>	Yes	2-aminoanthracene	10 ug
WP2 <i>uvrA</i>	No	methyl methanesulfonate	1000 ug

Plating and Toxicity Determination: Each test article extract was tested at seven dose levels. All dose levels were plated in triplicate. Test article extracts were serially diluted immediately before use.

Overlaid on plates containing bottom agar was the top agar mixture. The top agar mixture contained either:

- 1) 100 ul of tester strain and 100 ul of vehicle or undiluted test article extract, 500 ul of sham mix, and 2.0 ml molten selective top agar
- 2) 100 ul of tester strain, 100 ul of vehicle or undiluted test article extract, 500 ul of S-9 mix, and 2.0 ml molten top agar.

After the overlay had solidified, the plates were inverted and incubated for approximately 48 to 72 hours at $37 \pm 2^{\circ}\text{C}$. After the incubation period, the plates were evaluated and colonies counted. The condition of the bacterial background lawn was evaluated for evidence of test article extract toxicity relative to the vehicle control bacterial lawn. The presence of test article precipitate was also noted.

Plate Scoring: For each replicate plating, the mean and standard deviation of the number of revertants per plate was calculated. For the test article to be evaluated positive, its extract must cause a dose-related increase in the mean revertants per plate of a least one tester strain with a minimum of two increasing concentrations of test article extract. For strains TA1535 and TA1537, a data set is judged positive if the increase in mean revertants is equal to or greater than three times the mean vehicle control value. For strains TA98, TA100, and WP2 *uvrA*, the increase must be equal to or greater than two times the mean vehicle control value.

For a valid test, all *Salmonella* tester strain cultures must have an appropriate number of bacteria plated, demonstrate the correct genotype, and have the characteristic number of spontaneous revertants. The mean of each positive control must exhibit at least a three-fold increase in the number of revertants over the mean value of the respective vehicle control.

The saline and ethanol extracts of the test article Smooth Low Bleed Gel-filled Mammary Prosthesis did not increase the numbers of revertant colonies in any of the tester strains when in the presence or absence of microsomal enzymes, demonstrating a lack of mutagenic activity as measured by the Bacterial Reverse Mutation Assay.

Unscheduled DNA Synthesis in Mammalian Cells *In Vitro* Conducted With Test Article Extracts

A sterile finished device 275cc Smooth Low Bleed Gel-filled Mammary Prosthesis was evaluated for its saline and ethanol extract's potential to induce unscheduled DNA synthesis in primary cultures of rat hepatocytes. The testing was performed by BioReliance as a GLP study.

Test Article Preparation: A 275cc Smooth Low Bleed Gel-filled Mammary Prosthesis was extracted in saline and ethanol following USP extract ratio requirements. 0.2 grams test article per mL extraction medium was used. The extraction mixtures were shaken for 72 ± 2 hours at $50 \pm 2^\circ\text{C}$. Saline and ethanol extraction blanks were prepared in the same manner, without the addition of test article.

Test System: Primary rat hepatocytes derived from the liver of a normal adult male Sprague-Dawley rat were used in this study. After sacrifice, the animal was dissected and liver perfused. The liver was removed and cells were disassociated, counted, and seeded at 5×10^5 rat hepatocytes per 35 mm culture dish.

Positive Control: A solution of 7,12-Dimethylbenz(a)anthracene (7,12-DMBA) in dimethyl sulfoxide (DMSO) at 1 to 10 ug/ml.

Test Article Extract Controls: Vehicle controls consisted of 0.9% saline and 100% ethanol and were added to cell cultures at the same concentration as the highest test article extract concentration.

Study Design: Three replicate culture plates, each containing a 25 mm coverslip placed on the bottom, were seeded with 5×10^5 rat hepatocytes. Cell cultures were exposed to the appropriate undiluted test or control solution. Appropriate negative and positive control cultures were established. Each culture dish received ^3H -thymidine at a final concentration of 10 uCi/ml.

Cells were cultured for 18 - 24 hours to permit exposure of the test system to the test article extract or control solutions and allow thymidine incorporation into the DNA of the hepatocyte cells. Then the cells were washed, swelled in 1% sodium citrate and fixed in ethanol-glacial acetic acid fixative. The coverslips were air dried, mounted cell side up on glass slides, and allowed to dry. The slides were coated with Kodak NTB-2 emulsion (diluted 1:1 in deionized water) and stored in a refrigerator for seven days in light tight boxes with desiccant. Slides were developed in Kodak D-19 developer (diluted 1:1 in deionized water), fixed in Kodak fixer and stained in hematoxylin-eosin stain.

Cytotoxicity Determination: Lactate dehydrogenase (LDH) release associated with the cell cultures was evaluated to determine cytotoxicity of the test article extracts. At the time the replicate dose plates were established, four additional plates, without coverslips, were seeded with 5×10^5 rat hepatocytes. Two culture plates were treated with the test article extracts and two were treated with negative controls.

After the 18 - 24 hours exposure period, an aliquot of medium was removed from two dishes for each test article extract dose group and control group for LDH determination. In addition, the four plates that were seeded without coverslips were lysed with Triton X-100 to release 100% of the LDH in the cultures. Corrected LDH activities were obtained by subtracting the LDH activity of the solvent control cultures from the LDH activity of the test article extract cultures. Corrected LDH activity values for the test article extract cultures were compared to the corrected LDH activity of the 100% lysis control cultures.

The LDH activity assay values, presence of LDH activity dose-dependency and cell morphology observations were evaluated to determine if the test article extracts caused cytotoxicity. If observations of the fixed and stained cells indicate no obvious toxicity, elevated LDH values were considered artifacts.

Autoradiographic Scoring: Slides were read on an automated colony counter. Nuclear grains were counted in fifty cells in random areas on each of the three coverslips for each treatment and for each control. The net nuclear counts were determined by counting three nucleus-sized areas adjacent to each nucleus and subtracting the average cytoplasmic count from the nuclear count. Replicative synthesis was identified by nuclei completely blackened with grains and such cells were not counted.

Evaluation of Test Results: For each slide, the net nuclear counts were averaged and the standard deviation determined. The grand mean and standard deviation and the percent of cells in repair (cells with 5 or more nuclear grains) were determined. If the mean net nuclear count was increased by at least five

counts over the control, the results for a particular dose level were considered significant.

A test article was judged positive if it induced a significant increase in the average net nuclear grains when compared to that of the negative control. The test article was considered negative if no significant increase in the mean net nuclear grain counts was observed.

The test was considered valid because the positive control compound induced at least a 5 count increase in the mean net nuclear grain count over that in the negative controls. The proportion of cells in repair in the negative control was less than 15% and the net nuclear grain count of the solvent was less than one.

The saline and ethanol extracts of the test article Smooth Gel-filled Mammary Prosthesis did not induce a significant increase in unscheduled DNA synthesis in primary cultures of rat hepatocytes as measured by autoradiographic methods, demonstrating a lack of mutagenic activity associated with the mammary prosthesis.

Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells Conducted with Test Article Extracts

Saline and ethanol extracts of the test article, 275cc Smooth Low Bleed Gel-filled Mammary Prosthesis, were tested in the chromosome aberration assay using Chinese hamster ovary (CHO) cells. In the assay, one dose level (undiluted) of each test article extract was assessed in both the absence and presence of an Aroclor-induced S9-activation system. This testing was performed by BioReliance and was performed as a GLP study.

In the chromosome aberration assay, the cells were treated for 4 or 20 hours in the non-activated test system and for 4 hours in the S9-activated test system, and all groups were harvested at 20 hours after treatment initiation. The dose levels tested in the chromosome aberration assay were 100 $\mu\text{L}/\text{mL}$ for the saline extract and 10 $\mu\text{L}/\text{mL}$ for the ethanol extract. The saline and ethanol test article extracts were soluble in treatment medium at all concentrations tested. Toxicity (cell growth inhibition) for the saline extract was 5% and 2% at the dose level evaluated for chromosome aberrations, 100 $\mu\text{L}/\text{mL}$, in the non-activated 4 and 20 hour exposure groups, respectively. No toxicity (cell growth inhibition) was observed for the saline extract in the S9 activated 4 hour exposure group. No toxicity (cell growth inhibition) was observed in any of the three ethanol extract treatment groups.

Initially, the non-activated and S9-activated 4 hour exposure groups were scored for structural and numerical chromosome aberrations. No statistically significant increases in structural and numerical chromosome aberrations were observed in the non-activated or S9-activated 4 hour exposure groups relative

to the extraction blank control group, regardless of extract type ($p < 0.05$, Fischer's exact test).

Based on the findings of this study, saline and ethanol extracts of 275cc Smooth Low Bleed Gel-filled Mammary Prosthesis were concluded to be negative for the induction of structural and numerical chromosome aberrations in CHO cells.

MARKED GEL-FILLED MAMMARY PROSTHESIS TESTING

As part of the development work related to adding identification markings onto Mentor's Gel-filled Mammary Prostheses, biological testing of device patches with markings was performed (see reports HS33.020227.01 "Biocompatibility Report for Device Marked at the Patch Area" and HS33.020227.01 AdA "Addendum to the Biocompatibility Report for Device Marked at the Patch Area"). Gel-filled mammary prosthesis patches were subjected to the marking process. The patches were then submitted for testing. Since all Mentor Gel-filled Moderate Profile, Moderate Plus, and High Profile Prostheses use the same patch material and patch fabrication processes, testing one style patch adequately represents the patches of all three PMA product lines. The following summarizes the biological testing results.

ISO ELUTION METHOD (see HS33.020227.01)

marked patches were extracted in Minimal Essential Medium (MEM) for 24 hours at 37°C (47.5 cm² covered with 16 ml 1X MEM). Aliquots of MEM without the test material but subjected to the extraction conditions were used as the reagent controls. A vendor supplied high density polyethylene material was extracted in MEM as the negative control (30 cm² extracted in 10 ml MEM). Tin stabilized polyvinyl chloride was used as the positive control (60.1 cm² extracted in 20 ml of MEM). The test medium and controls were incubated with L-929 mouse fibroblast cells for 48 hours. All controls performed as expected. The test article extracts showed no evidence of causing cell lysis or toxicity; therefore, it met the requirement of the tests.

ISO ACUTE INTRACUTANEOUS REACTIVITY (see HS33.020227.01 and HS33.020227.01 AdA)

(Results from two different testing dates can be found in report HS33.020227.01. The first test showed that non-sterilized marked patch samples produced higher irritation scores than expected. Subsequent testing with sterilized marked patch samples produced acceptable scores. The higher than expected scores were attributed to the lack of sterilization. A third test, found in HS33.020227.01AdA and again using sterilized marked patches, also produced an acceptable score thereby providing verification that the non-sterile condition of the patch was associated with the irritation.)

[Report dated 2/8/02] Non-sterilized [REDACTED] marked patches were extracted in 0.9% sodium chloride USP solution (SC) and cottonseed oil, NF (CSO) for 24 hours at 70°C (47.5 cm² sample covered with 16 ml of the vehicle). Extraction vehicles without test article were similarly prepared and served as reagent controls. 0.2 ml doses of the test article extracts were injected intracutaneously into rabbits and observed for 24, 48, and 72 hours. Based upon the scoring results, there was no evidence of significant irritation from the SC extract, but the CSO extract showed evidence of moderate irritation. The Primary Irritation Index Characterization was 0.3 for the SC extract and 2.3 for the CSO extract.

[Report dated 4/15/02] Sterilized [REDACTED] marked patches were extracted in 0.9% sodium chloride USP solution (SC) and cottonseed oil, NF (CSO) for 24 hours at 70°C (45 cm² sample covered with 15 ml of the vehicle). The negative control was 38.5 cm² of sterilized [REDACTED] marked patch extracted in 13 ml of SC or CSO similar to the test article. 0.2 ml doses of the test article extracts were injected intracutaneously into rabbits and observed for 24, 48, and 72 hours. Based upon the scoring results, there was no evidence of significant irritation from the extracts. The Primary Irritation Index Characterization for the extracts was negligible.

[Report dated 7/9/02 in HS33.020227.01 AdA] Sterilized [REDACTED] marked patches were extracted in 0.9% sodium chloride USP solution (SC) and cottonseed oil, NF (CSO) for 24 hours at 70°C (24 cm² sample covered with 8 ml of the vehicle). Extraction vehicles without test article were similarly prepared and served as reagent controls. 0.2 ml doses of the test article extracts were injected intracutaneously into rabbits and observed for 24, 48, and 72 hours. Based upon the scoring results, there was no evidence of significant irritation from the extracts. The Primary Irritation Index Characterization for the extracts was negligible.

ISO ACUTE SYSTEMIC TOXICITY (see HS33.020227.01)

Non-sterilized [REDACTED] marked patches were extracted in 0.9% sodium chloride USP solution (SC) and cottonseed oil, NF (CSO) for 24 hours at 70°C (47.5 cm² sample covered with 16 ml of the vehicle). Extraction vehicles without test article were similarly prepared and served as reagent controls. Mice were injected with a test extract or control. SC was injected intravenously and CSO was injected intraperitoneally. The mice were observed at 0, 4, 24, 48, and 72 hours. The results indicated that mouse body weights were acceptable and there was no mortality. The animals appeared clinically normal except that the test and control mice injected with cottonseed oil appeared ungroomed 4 hours after dosing. This observation was considered an expected effect due to the unctuous nature of the extract. The test extracts showed no evidence of systemic toxicity. The test article extracts met the test requirement.

ISO SENSITIZATION STUDY (MAXIMIZATION METHOD) (see HS33.020227.01 AdA)

██████████ sterilized ██████████ marked patches were extracted in 0.9% sodium chloride USP solution (SC) and cottonseed oil, NF (CSO) for 24 hours at 70°C (18 cm² sample covered with 6 ml of the vehicle). Extraction vehicles without test article were similarly prepared and served as reagent controls. Following the standard protocol, the challenge sites were scored over a 72 hour period after challenge patch removal for erythema and edema. All animals were observed with the expected dermal reactions associated with intradermal injections of Freund's Complete Adjuvant. Otherwise, all animals appeared clinically normal throughout the test. Under the conditions of this study, the SC and CSO test article extracts showed no evidence of causing delayed dermal contact sensitization in the guinea pig.

████████████████████ DEVICE TESTING

Prior to Dow Corning withdrawing many of its silicone materials for use in long term implantable devices, Mentor used ██████████ and some elastomers to fabricate gel-filled mammary prostheses. Mentor also used ██████████ ██████████ for the gel-filled Low Bleed shells. Much of Mentor's more sophisticated and longer term biological testing was performed on these devices in the early to mid-1990's. Based upon FDA's Guidance for Manufacturers of Silicone Devices Affected by Withdrawal of Dow Corning Silastic Materials, the newer silicone vendors demonstrated the substantial equivalence of their replacement materials to the Dow Corning materials and Mentor demonstrated the equivalence of the finished devices made with both sets of materials. For that reason, the following finished device biological testing using ██████████ ██████████ materials is directly applicable to the Mentor Gel-filled Mammary Prostheses in this PMA.

IMMUNOLOGICAL EVALUATION**Low Bleed Gel-filled Mammary Prosthesis Shell -**

Studies have been performed to evaluate whether the smooth and Siltex Low Bleed shells from Mentor's Gel-filled Mammary Prostheses have an effect on the immunological system in mice. ██████████ performed these immunotoxicity/immunomodulation studies using methods based upon similar studies for silicone materials conducted under the auspices of the National Toxicology Program. Mentor's studies were performed in accordance with Good Laboratory Practices set forth by the FDA, 21 CFR 58. The full length reports for these immunotoxicity studies ("Immunological Evaluation of Smooth Envelope Silicone Elastomer in Female B6C3F1 Mice," "Immunological Evaluation of Smooth Envelope Silicone Elastomer (SESE) [Low Bleed Shell] Material In Female B6C3F1 Mice"

Protocol Number SESE-28-2-SC, "Immunological Evaluation of Smooth Envelope Silicone Elastomer (SESE) [Low Bleed Shell] Material In Female B6C3F1 Mice" Protocol Number SESE-10-1-SC, and "Immunological Evaluation of Textured Envelope Silicone Elastomer in Female B6C3F1 Mice") are located in the Biological Testing Appendices.

[Please note that the textured outer layer of Siltex Low Bleed Gel-filled shells is the same textured shell outer layer as that in Mentor's approved PMA for Siltex Saline-filled Mammary Prosthesis - see Mentor's Saline-filled Mammary Prosthesis PMA (#P940039/A7, Vol. V.C.3. Biological Testing Section, submitted May 4, 1995, No. 24 of 31, pp 7902 - 8027). In addition, the Smooth and Siltex Gel-filled Mammary Prostheses patches are both made from the same silicone material [REDACTED] as the textured layer of the shell.]

Immunological Evaluation of Smooth Envelope Silicone Elastomer in Female B6C3F1 Mice

Purpose: To determine the potential effects of Mentor's Smooth Low Bleed Gel-filled Mammary Prosthesis shell test article on the immune system. The parameters that were monitored are listed below:

- A. Body weight over time
- B. Terminal body weight and selected organ weights
- C. Hematology
- D. Leukocyte differentials
- E. Spleen IgM Antibody Response to a T-dependent Antigen
- F. T Cell and T Cell Subsets and B Cell Enumeration

(Note - the Low Bleed Patch information contained in the report is no longer of relevance because the patch material was subsequently changed [REDACTED] silicone. For that reason, only the smooth shell data are summarized here.)

In order to perform all of the above tests, the following animal implantation and sacrifice procedures were followed:

Test Article Preparation: Disks were punched from each of the smooth shells received from the study sponsor.

Test System/Study Groups: Three groups of female B6C3F1 mice were implanted subcutaneously with test article on Day 1 of the study with exposure to the implants occurring for 28 days. The Low Dose animals received a single implant with an approximate area of 14.13 mm². The Middle Dose animals received a single implant with an approximate area of 28.26 mm². The High Dose animals received two implants, each with an approximate area of 28.26 mm² for a total area of 56.52 mm². (The size of the implanted disc was based upon procedures

developed in previous National Toxicology Program studies and was the largest size of a single implant that would not interfere with the normal movement and activity of the recipient mice.) One group of mice served as Naive Control animals and did not undergo any surgical procedures. Another group of mice, serving as the Sham Control animals, underwent the same procedures as the High Dose animals except no implants were inserted. Other mice served as the Positive Control animals and received an appropriate chemical solution (cyclophosphamide) of known immunotoxic activity.

Body Weights: Each animal was weighed on the first day of the study and on days 1, 8, 15, 22, and 29. Body weights and body weight changes were compared between the animal groups.

Organ Weights: After sacrifice, the thymus and spleen were removed from the animals, cleaned of connective tissue and weighed. The organ weights, expressed in milligrams and as percent of body weight, were compared between the animal groups.

Hematologic Parameters: Prior to sacrifice, blood was collected from the mice. Erythrocyte and leukocyte numbers, leukocyte differentials, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined. Values were compared between the animal groups.

Assay Procedures: The assay procedures performed to evaluate immunotoxicity and immunomodulation are described below:

A. Spleen IgM Antibody Response to a T-dependent Antigen, Sheep Erythrocytes (Day 4 Response):

Purpose: Sheep red blood cells (sRBC) are a T-dependent antigen requiring T cells, B cells and macrophages to function properly to obtain an antibody forming cell (AFC) response. If a test article affects these cell types, an altered response will be observed.

Procedure: Mice were implanted with test article on day 1 of the study and on day 25 they were injected with sRBC. On day 29 (peak day of IgM response), the spleen from each animal was removed, and the spleen cells added to test tubes containing guinea pig complement, sRBC and warm agar. An aliquot of each preparation was plated onto separate petri dishes and incubated for 3 hours. Following incubation, the plates were evaluated for plaque formation.

A plaque, occurring from the lysis of sRBC, is elicited as a result of the interaction of complement and antibodies which are directed against sheep erythrocytes and produced in response to the intravenous sensitization. Each plaque is generated from a single IgM antibody-producing B cell and, thus, the number of AFC present in the whole spleen can be calculated. The data were expressed as specific activity (AFC/10⁶ spleen cells) and AFC per spleen. Results were compared between the animal groups to determine if the presence of the test article caused an effect.

B. T Cell and T Cell Subsets and B Cell Enumeration:

Purpose: The number of T and B lymphocytes in the spleens of mice exposed to test article provide data for functional studies. If the test article affects the cell numbers, an altered immune response may be observed.

Procedure: Mice were exposed to the implanted test article for 28 days. On the 29th day, the spleen from each animal was removed and separate splenocyte suspensions prepared. The lymphocytes were incubated in fluorescent stain solutions to interact with specific cell surface markers. The resulting cell fluorescence was analyzed with a Becton Dickinson FACScan Flow Cytometer (FACScan) to determine the number of B lymphocytes (Ig⁺), and the number of cells present in the T cell subsets (CD4⁺ and CD8⁺).

To determine the number of T cells, cell suspensions were stained with an antimouse Thy 1.2 monoclonal antibody conjugated to fluorescein isothiocyanate (FITC). To determine the number of B lymphocytes, a FITC conjugated affinity purified anti-mouse immunoglobulin was used. For T cell subsets, two stains were employed:

- (1) phycoerythrin (PE) conjugated to monoclonal antibody specific for the L3T4 cell surface protein marker, used to identify CD4⁺ cells, and
- (2) FITC conjugated monoclonal antibody specific for the Lyt-2 cell surface protein marker, used to identify CD8⁺ cells.

After a 30 minute stain incubation period, the cells were washed, and resuspended in a propidium iodide (PI) solution. PI is a DNA specific fluorescent stain and indicator of dead cells. After a 5 minute incubation, the cells were washed, resuspended in an appropriate buffer solution and analyzed by the FACScan. The FACScan operating

parameters were set to eliminate evaluation of dead cells and red blood cells.

Based on evaluating 5000 cells for each sample, the number of cells which stained positively for each antibody was determined. Results were compared between the animal groups to determine if the presence of the test article caused an effect on the number of each evaluated cell type.

Exposure to the Smooth Gel-filled Mammary Prosthesis shell disc(s) did not result in significant changes in body weight, spleen weight, red blood cell number, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, leukocyte number, or leukocyte differential. A dose dependent decrease in the thymus weight was noted.

Exposure to the Smooth Gel-filled Mammary prosthesis shell disc(s) did not result in biologically significant changes in the antibody-forming cell response to the T-dependent antigen, sheep erythrocytes. There were no significant effects on the number of cells stained positive as CD4⁺ and CD8⁺ cells or B cells. There was a dose dependent increase in the number of Thy 1.2⁺ T cells noted.

In summary, even with the indicated alterations in immune parameters, decreased thymus weight and increased number of Thy 1.2⁺ cells in the spleen, animals exposed to the Smooth Low Bleed shell responded similarly to control animals in their ability to produce antibodies to the T-dependent antigen sheep erythrocytes. This complex immune response requires T cells, B cells, and macrophages to function properly in concert in order to successfully produce antibody-forming cells. An additional study was performed to better understand the increase in T cells noted in this study.

Immunological Evaluation of Smooth Envelope Silicone Elastomer (SESE) [Low Bleed] Material In Female B6C3F1 Mice, Protocol Number SESE-28-2-SC -

Purpose: To obtain an in-depth understanding of the results obtained in the above immunotoxicological study conducted using Mentor's Smooth Low Bleed Gel-filled Mammary Prosthesis shell material, particularly the increase in the number of Thy 1.2⁺ cells in the spleen.

Test Article Preparation: Disks were punched from each of the smooth shells received from the study sponsor.

Test System/Study Groups: Three groups of female B6C3F1 mice were implanted subcutaneously with test article on Day 1 of the study with

exposure to the implants occurring for 28 days. The Low Dose animals received a single implant with an approximate area of 14.13 mm². The Middle Dose animals received a single implant with an approximate area of 28.26 mm². The High Dose animals received two implants, each with an approximate area of 28.26 mm² for a total area of 56.52 mm². One group of mice served as Naive Control animals and did not undergo any surgical procedures. Another group of mice, serving as the Sham Control animals, underwent the same procedures as the High Dose animals except no implants were inserted. Other mice served as the Positive Control animals and received an appropriate chemical solution (cyclophosphamide) of known immunotoxic activity.

Body Weights: Each animal was weighed on the first day of the study and on days 1, 8, 15, 22, and 29. Body weights and body weight changes were compared between the animal groups.

Organ Weights: After sacrifice, the thymus and spleen were removed from the animals, cleaned of connective tissue and weighed. The organ weights, expressed in milligrams and as percent of body weight, were compared between the animal groups.

T Cell and T Cell Subsets and B Cell Enumeration: The markers to be used and the cell types to be identified are indicated below (a Tier II assay used by the National Toxicology Program¹):

MARKERS	CELL TYPE IDENTIFIED
CD4 ⁺	T Helper
CD8 ⁺	T Suppressor/Cytotoxic T Lymphocyte
CD3 ⁺	Pan T Cells
Thy 1.2 ⁺	Pan T Cells (and other cells)
Ig ⁺	Pan B Cells (and other cells)
B220 ⁺	Pan B Cells
CD4 ⁺ CD8 ⁺	Immature T Cells
Thy 1.2 ⁺ CD3 ⁺	T Cells with Thy 1.2 marker
Thy 1.2 ⁺ B220 ⁺	B Cells with Thy 1.2 marker
Thy 1.2 ⁺ Ig ⁺	B Cells (and other cells)with Thy 1.2 marker

¹Luster, M.I., A.E. Munson, P. Thomas, M.P. Holsapple, J. Fenters, K.L. White, Jr., L.D. Lauer, and J.H. Dean. 1988. Development of a testing battery to access chemical-induced immunotoxicity. Fund. Appl. Toxicol. 10:2-19.

Based on evaluating 5000 cells for each sample, the number of cells staining positively was determined. Results were compared between the animal groups to determine if the presence of the test article caused an effect on the number of each evaluated cell type.

The results showed that exposure to the Smooth Low Bleed shell material did not result in significant changes in body weight, changes in body weight gain or spleen weight. A decrease (19%) was observed in thymus weight of animals of the middle dose group which received the smooth shell implants. However, this decrease was only statistically significant when the data were expressed as absolute weight. When the data were expressed as percent of body weight, the more appropriate way to present the data, the decrease in thymus weight was not statistically different from the sham controls.

In addition, the results showed that exposure to the Smooth Low Bleed shell material did not result in significant changes in spleen cell number, the number of Ig⁺ or B220⁺ B cells, CD4⁺ T Helper cells, CD8⁺ T Suppressor/Cytotoxic T Lymphocyte cells, CD4⁺CD8⁺ immature T cells or the CD4⁺/CD8⁺ ratio. Animals exposed to the low dose only of the smooth shell material had an increase (22%) in the number of T cells as enumerated by the CD3 marker but this increase is not considered biologically significant.

As in the first study above, a dose-dependent increase in Thy 1.2⁺ cells was observed in the spleen of animals implanted with the smooth shell material. However, dual antibody marker studies clearly demonstrated that the increase in Thy 1.2⁺ cells was not associated with an increase in B cells, nor was it associated with an increase in T cells. Since neither B cells nor T cells account for the increase in Thy 1.2⁺ cells, the increase may be due to an increase in a "non-immune" cell type. Several "non-immune" cell types have been reported to display the Thy 1.2 marker including neurons, epithelia cells, and fibroblasts. Since fibroblasts would be expected to participate in the formation of and maintenance of a capsule surrounding any type of implant, fibroblasts would be the most likely candidate of the "non-immune" cell types to account for the increase in Thy 1.2⁺ cells in the spleens. Accordingly, the increase in Thy 1.2⁺ cells would not be considered to represent an adverse effect on the immune response.

Therefore, the results from this protocol study, taken in conjunction with the lack of effect on the functional IgM antibody response to the T-dependent antigen, sheep erythrocytes, assay conducted in the above protocol study, demonstrate that under the experimental conditions used exposure to the Smooth Gel-filled Mammary Prosthesis shell material did not adversely affect the immune response.

Immunological Evaluation of Smooth Envelope Silicone Elastomer (SESE) [Low Bleed] Material In Female B6C3F1 Mice, Protocol Number SESE-10-1-SC

Purpose: To determine whether there were any adverse effects on the functional ability of thymus-derived T cells following exposure to Mentor's Smooth Low Bleed Gel-filled Mammary Prosthesis shell material.

Test Article Preparation: Disks were punched from each of the smooth shells received from the study sponsor.

Test System/Study Groups: Three groups of female B6C3F1 mice were implanted subcutaneously with test article on Day 1 of the study with exposure to the implants occurring for 10 days. The Low Dose animals received a single implant with an approximate area of 14.13 mm². The Middle Dose animals received a single implant with an approximate area of 28.26 mm². The High Dose animals received two implants, each with an approximate area of 28.26 mm² for a total area of 56.52 mm². One group of mice served as Naive Control animals and did not undergo any surgical procedures. Another group of mice, serving as the Sham Control animals, underwent the same procedures as the High Dose animals except no implants were inserted. Other mice served as the Positive Control animals and received an appropriate chemical solution (cyclophosphamide) of known immunotoxic activity.

Body weight measurements: Animals were weighed on the first day of treatment and on day 11, the day of sacrifice. (A blood sample was also taken on day 11 for antibody testing in a separate study summarized in the next section below.)

Organ weights: After sacrifice, the thymus and spleen were removed from the animals, cleaned of connective tissue, weighed, and a thymus sample preserved for histopathology. The organ weights, expressed in milligrams and as percent of body weight, were compared between the animal groups.

Spleen cell proliferation response to the T cell mitogens concanavalin A (Con A) and phytohemagglutinin (PHA): Blastogenesis (cellular enlargement with increase in endoplasmic reticulum) and proliferation (increase in DNA synthesis and cell division) are integral parts of the immune response of both B and T cells. If a drug or chemical adversely affects these responses, an altered immune response can result. A significant change in the spleen cell proliferative response to mitogens, when appropriately compared to untreated controls, indicates that the test agent is capable of modulating blastogenesis and/or proliferation and, thus, has the potential for modifying the immune system. Spleen cells exposed to the shell material for 10 days were isolated and then exposed to Con A and PHA. Using ³H-thymidine during an 18 hour incubation, cell proliferation is assessed using a liquid scintillation counter.

Mixed leukocyte response (MLR) to DBA/2 spleen cells: MLR is a more sensitive assay than the spleen cell proliferative response to

mitogens and is a sensitive indicator of effects on cell-mediated immunity. A significant change in the MLR, when appropriately compared to untreated controls, indicates that the test agent is capable of modulating recognition and/or proliferation in response to stimulation with allogeneic cells. Agents which produce such effects have the potential for modifying the immune system. Spleen cells exposed to the shell material for 10 days were isolated and then exposed to Mitomycin C treated DBA/2 spleen cells for 5 days. During the last 18 hours, ³H-thymidine is added. Cell proliferation is assessed using a liquid scintillation counter and from that a stimulation index is derived.

In animals implanted with the smooth shell material, no effects were observed on body weight, body weight gain, spleen weight (absolute or percent body weight), thymus weight (absolute or percent body weight), histopathology of the thymus, spleen cell proliferative response to the T cell mitogens concanavalin A or phytohemagglutinin, and the mixed leukocyte response.

The results of this third protocol study, taken in conjunction with the lack of effect on the IgM antibody-forming cell responses and cell surface marker studies conducted in the previous two protocol studies, demonstrate that under the experimental conditions used exposure to Mentor's Smooth Gel-filled Mammary Prosthesis shell material does not adversely affect the immune response.

Immunological Evaluation of Textured Envelope Silicone Elastomer in Female B6C3F1 Mice [Note - this report also contains testing data on Becker Valve materials; however, those materials are of no relevance to this PMA. For that reason, only the textured shell data are summarized below.]

Purpose: To determine the potential effects of Mentor's Siltex textured Low Bleed Gel-filled Mammary Prosthesis shell test article on the immune system. The parameters to be monitored are listed below:

- A. Body weight over time
- B. Terminal body weight and selected organ weights
- C. Hematology
- D. Leukocyte differentials
- E. Spleen IgM Antibody Response to a T-dependent Antigen
- F. T Cell and T Cell Subsets and B Cell Enumeration

In order to perform all of the above tests, the following animal implantation and sacrifice procedures were followed:

Test Article Preparation: Disks were punched from each of the textured shells received from the study sponsor.

Test System/Study Groups: Three groups of female B6C3F1 mice were implanted subcutaneously with test article on Day 1 of the study with exposure to the implants occurring for 28 days. The Low Dose animals received a single implant with an approximate area of 14.13 mm². The Middle Dose animals received a single implant with an approximate area of 28.26 mm². The High Dose animals received two implants, each with an approximate area of 28.26 mm² for a total area of 56.52 mm². One group of mice served as Naive Control animals and did not undergo any surgical procedures. Another group of mice, serving as the Sham Control animals, underwent the same procedures as the High Dose animals except no implants were inserted. Other mice served as the Positive Control animals and received an appropriate chemical solution (cyclophosphamide) of known immunotoxic activity.

Body Weights: Each animal was weighed on the first day of the study and on days 1, 8, 15, 22, and 29. Body weights and body weight changes were compared between the animal groups.

Organ Weights: After sacrifice, the thymus and spleen were removed from the animals, cleaned of connective tissue and weighed. The organ weights, expressed in milligrams and as percent of body weight, were compared between the animal groups.

Hematologic Parameters: Prior to sacrifice, blood was collected from the mice. Erythrocyte and leukocyte numbers, leukocyte differentials, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined. Values were compared between the animal groups.

Assay Procedures: The assay procedures performed to evaluate immunotoxicity and immunomodulation are described below:

A. Spleen IgM Antibody Response to a T-dependent Antigen, Sheep Erythrocytes (Day 4 Response):

Purpose: Sheep red blood cells (sRBC) are a T-dependent antigen requiring T cells, B cells and macrophages to function properly to obtain an antibody forming cell (AFC) response. If a test article affects these cell types, an altered response will be observed.

Procedure: Mice were implanted with test article on day 1 of the study and on day 25 they were injected with sRBC. On day 29 (peak day of IgM response), the spleen from each animal was removed, and the spleen cells added to test tubes containing guinea pig complement,

sRBC and warm agar. An aliquot of each preparation was plated onto separate petri dishes and incubated for 3 hours. Following incubation, the plates were evaluated for plaque formation.

A plaque, occurring from the lysis of sRBC, is elicited as a result of the interaction of complement and antibodies directed against sheep erythrocytes and produced in response to the intravenous sensitization. Each plaque is generated from a single IgM antibody-producing B cell and, thus, the number of AFC present in the whole spleen can be calculated. The data were expressed as specific activity (AFC/10⁶ spleen cells) and AFC per spleen. Results were compared between the animal groups to determine if the presence of the test article caused an effect.

B. T Cell and T Cell Subsets and B Cell Enumeration:

Purpose: The number of T and B lymphocytes in the spleens of mice exposed to test article provide data for functional studies. If the test article affects the cell numbers, an altered immune response may be observed.

Procedure: Mice were exposed to the implanted test article for 28 days. On the 29th day, the spleen from each animal was removed and separate splenocyte suspensions prepared. The lymphocytes were incubated in fluorescent stain solutions to interact with specific cell surface markers. The resulting cell fluorescence was analyzed with a Becton Dickinson FACScan Flow Cytometer (FACScan) to determine the number of B lymphocytes (Ig⁺), and the number of cells present in the T cell subsets (CD4⁺ and CD8⁺).

To determine the number of T cells, cell suspensions were stained with an antimouse Thy 1.2 monoclonal antibody conjugated to fluorescein isothiocyanate (FITC). To determine the number of B lymphocytes, a FITC conjugated affinity purified anti-mouse immunoglobulin was used. For T cell subsets, two stains were employed:

- (1) phycoerythrin (PE) conjugated to monoclonal antibody specific for the L3T4 cell surface protein marker, used to identify CD4⁺ cells, and
- (2) FITC conjugated monoclonal antibody specific for the Lyt-2 cell surface protein marker, used to identify CD8⁺ cells.

After a 30 minute stain incubation period, the cells were washed, and resuspended in a propidium iodide (PI) solution. PI is a DNA specific

fluorescent stain and indicator of dead cells. After a 5 minute incubation, the cells were washed, resuspended in an appropriate buffer solution and analyzed by the FACScan. The FACScan operating parameters were set to eliminate evaluation of dead cells and red blood cells.

Based on evaluating 5000 cells for each sample, the number of cells staining positively for each antibody was determined. Results were compared between the animal groups to determine if the presence of the test article caused an effect on the number of each evaluated cell type.

Exposure to the Siltex Low Bleed shell disc(s) did not result in significant changes in body weight, body weight gain, thymus weight, spleen weight, red blood cell number, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, or leukocyte number. A slight decrease in mean corpuscular hemoglobin was observed with the middle dose of Siltex shell but this was not dose dependent and no other erythroid parameters were affected; therefore, this was not considered to be biologically significant. In the middle dose Siltex shell animals, an increase in eosinophils was observed in the leukocyte differential. However, the increase was not dose dependent and not considered to be related to the test material.

Exposure to the Siltex Gel-filled Mammary Prosthesis shell disc(s) did not result in biologically significant changes in the antibody-forming cell response to the T-dependent antigen, sheep erythrocytes. There was a slight decrease in B cells (Ig^+) for the high dose animals; however, the response was not dose dependent. There were no significant effects on the number of T cells ($Thy 1.2^+$) in the spleen and the number of cells stained positive as $CD4^+$ and $CD8^+$ cells.

In summary, while there were slight decreases in the number of B cells (Ig^+) in the high dose animals with Siltex Low Bleed shells, the functional ability of the animals to produce antibodies to the T-dependent antigen sheep erythrocytes was not compromised. This suggests that the Siltex Low Bleed shell did not significantly alter the immune system, including the lack of effect on T cells, and T cell subsets, leukocyte number, spleen weight, spleen cellularity, and thymus weight.

Low Bleed Gel-filled Mammary Gel

Studies have been performed to evaluate the potential effects of gel from Gel-filled Mammary Prostheses on the immune system in mice. The National Toxicology Program (Michael Luster, Ph.D., National Institute of Environmental Health Sciences) sponsored studies at the Medical College of Virginia (MCV) to determine the potential effects of silicone (including gel from a Dow Corning Silastic II Mammary Implant

which is made from gel kit parts Q7-2167 and Q7-2168 combined in a 3:1 ratio) on the immune system and host resistance to selected microbial and tumor models. The full-length reports entitled "Immunotoxicity of Silicone in Female B6C3F1 Mice - 10 Day Exposure" and "Immunotoxicity of Silicone in Female B6C3F1 Mice - 180 Day Exposure" can be found in the Biological Testing Appendices.

[Please note that the gel in Dow Corning's devices used for this immunological testing

[REDACTED] As a result, the immunological testing described here for Dow Corning gel is directly applicable to Mentor's silicone gel filler.]

Immunotoxicity of Silicone in Female B6C3F1 Mice - 10 Day Exposure

One ml of gel, approximately equal to 1/20th the body weight of the mouse, was taken from a Dow Corning breast implant for testing. The ten day study exposure period was selected because it was the peak time of the initial inflammatory response.

An objective of the toxicology studies was to characterize the toxicological profile produced by treatment with silicone gel (see Table 1 below). Subcutaneous implantation of the silicone, including gel, produced no significant changes in any of the parameters measured. There was no implant-related mortality from exposure and no overt signs of toxicity. There were no gross pathologic findings at the time of necropsy. For the most part, body weight and body weight gain were not altered by the implants. The hematologic and serum chemistry parameters measured were not different from the vehicle control group (except for SGPT and serum globulin levels, but they were within the historical control range of the Medical College of Virginia, Immunotoxicology Program). Body weight was not adversely affected by the silicone implant. There were no significant changes in the weight of the brain, liver, spleen, thymus, lungs or kidneys. Bone marrow cellularity, CFU-M and CFU-GM stem cells were unaffected by the implanted material.

The immunological results are summarized in Table 2 below. Implantation of mice with silicone preparations (including gel) produced no effects on indicators of humoral immunity, such as changes in B cell number, proliferative ability or ability to differentiate into antibody-producing cells to a T-dependent antigen. Indicators of cell-mediated immunity were also unaffected as seen in a lack of effect on T lymphocyte numbers and subset analysis, and spleen cell response to the T-dependent antigen, indicating that the regulatory T cells were similar to those of control mice. Proliferative capacity, as measured by response to T cell mitogens and to allogenic cells,

was unaffected by silicone implants including gel. Differentiation and the killing mechanism of T cells were intact as seen in a CTL response. Indications of innate immunity that were unaffected included macrophage numbers and function, natural killer cell activity and complement activity.

Three host resistance study results are summarized in Table 3 below. Implantation of mice with the silicone gel did not produce any changes in host resistance to two bacterial models or one tumor host resistance model.

Table 1
SUMMARY TABLE FOR TOXICOLOGY STUDIES
10-DAY STUDIES (SIL-10-1-SC)
Results

Parameter	Gel	Comments
General		
Body Weight	7% Incr	
Gross Pathology	No Effect	
Organ Weights		
Liver	11% Decr	% Body Wt
Spleen	No Effect	
Lungs	No Effect	
Thymus	No Effect	
Kidney	No Effect	
Brain	6% Decr	Not Biologically Significant
Hematology		
RBCs	No Effect	
Retics	No Effect	
Leukocytes	No Effect	
Leukocyte Diff	No Effect	
Total		
Lymphocytes	No Effect	
Serum Chemistries		
SGPT	32% Decr	
Albumin	No Effect	
BUN	No Effect	
Glucose	No Effect	
Total Protein	No Effect	
Globulin	17% Decr	
Bone Marrow		
Cellularity	No Effect	
DNA Synthesis	No Effect	
Stem Cells	No Effect	
CFU-GM	No Effect	
CFU-M	No Effect	

Table 2
SUMMARY TABLE FOR IMMUNOLOGY STUDIES
10-DAY STUDIES (SIL-10-1-SC)

Parameter	Results	
	GEL	Comments
	% Value	
B Cells	No Effect	
T Cells	No Effect	
CD4 ⁺ CD8 ⁻	No Effect	
CD4 ⁻ CD8 ⁺	No Effect	
CD4 ⁺ CD8 ⁺	No Effect	
CD4 ⁻ CD8 ⁻	No Effect	
	Absolute Value	
B Cells	No Effect	
T Cells	No Effect	
CD4 ⁺ CD8 ⁻	No Effect	
CD4 ⁻ CD8 ⁺	No Effect	
CD4 ⁺ CD8 ⁺	No Effect	
CD4 ⁻ CD8 ⁻	No Effect	
	Proliferation Assays	
Con A	No Effect	
PHA	No Effect	
LPS	No Effect	
Medium	No Effect	
F(ab' ₂) + BSF-1	No Effect	
	Cell-Mediated Immunity	
MLR	No Effect	
CTL	No Effect	
	Innate Immunity	
NK Cell Activity	No Effect	
RES Function	No Effect	
cRBC	No Effect	
Phagocytosis		
P.E. Differential	No Effect	
P.E. Cell Macrophage Activation w/Gamma Interferon	32% Decr	Not Biologically Significant
Covasphere Phagocytosis	No Effect	

Table 3

SUMMARY TABLE FOR HOST RESISTANCE STUDIES
10-DAY STUDIES

SIL-10-1-SC

Results

Parameter	GEL	Comments
<i>Listeria monocytogenes</i>	No Effect	
<i>Streptococcus pneumoniae</i>	No Effect	
B16F10	No Effect	

Immunotoxicity of Silicone in Female B6C3F1 Mice - 180 Day Exposure

One ml of gel, approximately equal to 1/20th the body weight of the mouse, was taken from a Dow Corning breast implant for testing. The 180 day study exposure period was selected because it provides for evaluation of the immune system during the chronic phase where the capsule is well formed.

An objective of this toxicology study was to characterize the toxicological profile produced by treatment with silicone gel (see Table 1 below). Subcutaneous implantation of the silicone material produced no significant changes in any of the parameters measured. There was no implant-related mortality from exposure and no overt signs of toxicity. There were no gross pathologic findings at the time of necropsy. For the most part, body weight and body weight gain were not altered by the implants. The hematologic and serum chemistry parameters measured were not different from the vehicle control group and were within the historical control range of the Medical College of Virginia, Immunology Program. Body weight was not adversely affected by the silicone implant. There were no significant changes in the weight of the brain, liver, spleen, thymus, lungs, or kidneys. Bone marrow cellularity, CFU-M and CFU-GM stem cells were unaffected by the implant material.

The immunological results are summarized in Table 2 below. Implantation of mice with silicone preparations (including gel) produced no effects on indicators of humoral immunity, such as changes in B cell number, proliferative ability or ability to differentiate into antibody-producing cells to a T-dependent antigen. Indicators of cell-mediated immunity were also unaffected as seen in a lack of effect on T lymphocyte numbers and subset analysis, and spleen response to the T-dependent antigen, indicating that the regulatory T cells were

similar to those of control mice. Proliferative capacity, as measured by response to T cell mitogens and to allogeneic cells, was unaffected by silicone gel material. Differentiation and the killing mechanism of T cells were intact as seen in a CTL response. Indicators of innate immunity that were unaffected included macrophage numbers and function, natural killer cell activity and complement activity. Changes in natural killer cell function were slightly decreased by gel, but after performing dose response and time course studies, it was concluded that the natural killer cell activity was minimally affected, lacked consistency, and is not of a magnitude that warrants discontinued use in implant materials.

Three host resistance study results are summarized in Table 3 below. Implantation of mice with the silicone gel did not produce any changes in host resistance to two bacterial models or one tumor host resistance model.

Table 1

SUMMARY TABLE FOR TOXICOLOGY STUDIES
180-DAY STUDY (SIL-180-1-SC)

Parameter	Results	
	GEL	Comments
General		
Body Weight	No Effect	
Gross Pathology	No Effect	
Organ Weights		
Liver	No Effect	
Spleen	No Effect	
Lungs	No Effect	
Thymus	No Effect	
Kidney	No Effect	
Brain	No Effect	
Hematology		
RBCs	No Effect	
Retics	No Effect	
Leukocytes	No Effect	
Leukocyte Diff	No Effect	
Serum Chemistries		
SGPT	No Effect	
Albumin	No Effect	
BUN	No Effect	
Glucose	No Effect	
Total Protein	No Effect	
Globulin	No Effect	
Bone Marrow		
Cellularity	No Effect	
DNA Synthesis	No Effect	
Stem Cells	No Effect	
CFU-GM	No Effect	
CFU-M	22% Inc	Per 10 ⁵ cells

Table 2
SUMMARY TABLE FOR IMMUNOLOGY STUDIES
180-DAY STUDY (SIL-180-1-SC)

Parameter	GEL	Comments
Cell Surface Markers (Percent Value)		
B Cells	12% Decr	Studies Repeated for Dose Response
CD4 ⁺ CD8 ⁻	22% Incr	Studies Repeated for Dose Response
CD4 ⁻ CD8 ⁺	45% Incr	Studies Repeated for Dose Response
CD4 ⁺ CD8 ⁺	95% Incr	Studies Repeated for Dose Response
Cell Surface Markers (Absolute Number)		
B Cells	18% Decr	Studies Repeated for Dose Response
CD4 ⁺ CD8 ⁻	68% Incr	Studies Repeated for Dose Response
CD4 ⁻ CD8 ⁺	76% Incr	Studies Repeated for Dose Response
CD4 ⁺ CD8 ⁺	200% Incr	Studies Repeated for Dose Response

Repeated with Dose Response the GEL Material
Cell Surface Markers (Absolute Number)

Parameter	VH	0.5 ml	1.0 ml	2.0 ml	Comments
B Cells	No Effect	No Effect	No Effect	No Effect	
CD4 ⁺ CD8 ⁻	No Effect	No Effect	No Effect	No Effect	Repeat Study
CD4 ⁻ CD8 ⁺	No Effect	No Effect	No Effect	No Effect	Dose
CD4 ⁺ CD8 ⁺	No Effect	No Effect	No Effect	No Effect	Response

Parameter	GEL	Comments
IgM AFC to sRBC (T-dependent) Day 4 IgM		
PFC/10 ⁶	No Effect	
PFC/Spleen	No Effect	
IgM AFC to sRBC (T-dependent) Day 5 IgM		
PFC/10 ⁶	No Effect	

Table 2 (continued)
SUMMARY TABLE FOR IMMUNOLOGY STUDIES
180-DAY STUDY (SIL-180-1-SC)

Spleen Cell Proliferative Response to Mitogens and Allogenic Cells

Stimulus	GEL	Comments
ConA	No Effect	
PHA	No Effect	
LPS	No Effect	
Medium	No Effect	
F(ab') ₂ + BSF-1	No Effect	
MLR	No Effect	

Cytotoxic T Cell Response

Parameter	GEL	Comments
Lysis	No Effect	

Natural Killer Cell Response

Parameter	GEL	Comments
NK Cell Activity	35% Decr	Dose-Response Study to be Performed

Repeated (180 Days) with Dose Response of the GEL Material

Parameter	VH	1.0 ml	2.0 ml	3.0ml	Comments
NK Cell Activity	No Effect	No Effect	No Effect	53% Decr	Dose-Response Study Performed

Time Course Studies
Repeated with Dose Response of the GEL Material at 30 Days

Parameter	VH	1.0 ml	2.0 ml	3.0ml	Comments
NK Cell Activity	No Effect	No Effect	No Effect	23% Decr	Dose-Response Study Performed

Table 2 (continued)
SUMMARY TABLE FOR IMMUNOLOGY STUDIES
180-DAY STUDY (SIL-180-1-SC)

Time Course Studies
Repeated with Dose Response of the GEL Material at 60 Days

Parameter	VH	1.0 ml	2.0 ml	3.0ml	Comments
NK Cell Activity	No Effect	No Effect	No Effect	No Effect	Dose-Response Study Performed

Time Course Studies
Repeated with Dose Response of the GEL Material at 90 Days

Parameter	VH	1.0 ml	2.0 ml	3.0ml	Comments
NK Cell Activity	No Effect	No Effect	No Effect	23% Decr	Dose-Response Study Performed

Time Course Studies
Repeated with Dose Response of the GEL Material at 180 Days

Parameter	VH	1.0 ml	2.0 ml	3.0ml	Comments
NK Cell Activity	No Effect	No Effect	No Effect	28% Decr	Dose-Response Study Performed

Serum Complement

Parameter	GEL	Comments
CH 50	No Effect	
C3	No Effect	

Table 2 (continued)
SUMMARY TABLE FOR IMMUNOLOGY STUDIES
180-DAY STUDY (SIL-180-1-SC)

Macrophage Functions:

Functional Activity of Reticuloendothelial System

Parameter	GEL	Comments
Vascular Clearance	No Effect	
Liver Uptake	26% Incr	
Spleen Uptake	No Effect	
Repeated with Dose Response of the GEL Material: No Effect on Liver Uptake		

Covasphere Phagocytosis

Parameter	GEL	Comments
Phagocytosis	No Effect	

Chicken Erythrocyte Phagocytosis

Parameter	GEL	Comments
Phagocytosis	No Effect	

Peritoneal Cell Differential

Parameter	GEL	Comments
% Lymph's	No Effect	
Absol Mono's	26% Decr	

Table 3
SUMMARY TABLE FOR HOST RESISTANCE STUDIES
180-DAY STUDY (SIL-180-1-SC)

Results

Model	GEL	Comments
<i>Listeria monocytogenes</i>	No Effect	
<i>Streptococcus pneumoniae</i>	No Effect	
B16F10	No Effect	

EVALUATION OF TISSUE ANTIBODIES TO GEL-FILLED MAMMARY SHELL

In conjunction with [REDACTED] immunological evaluation of Smooth Low Bleed Gel-filled Mammary shell material (described above in "Immunological Evaluation of Smooth Envelope Silicone Elastomer (SESE) [Low Bleed] Material In Female B6C3F1 Mice, Protocol Number SESE-10-1-SC."), mice which had been implanted for 10 days with discs of smooth shell material had their serum evaluated for the presence of tissue autoantibodies. This study, entitled "Determination of Tissue Antibodies in Serum from Mice Implanted with Silicone Elastomer Discs" was performed at [REDACTED]

[REDACTED] (See the Biological Testing Appendices for the complete report.) The study was performed in accordance with the guidelines established by [REDACTED].

Purpose: To evaluate the sera of mice subcutaneously implanted with silicone elastomer shell material discs from Mentor Smooth Gel-filled Mammary Prostheses for the presence of tissue autoantibodies and the level of cytokines.

Background Information: Serum was obtained from mice at the time of sacrifice in the previously discussed immunotoxicity study entitled "Immunological Evaluation of Smooth Envelope Silicone Elastomer (SESE) [Low Bleed] Material In Female B6C3F1 Mice, Protocol Number SESE-10-1-SC." Three groups of B6C3F1 strain mice had been implanted subcutaneously with test article [disk(s) of smooth gel-filled mammary shell] on Day 1 of the study with exposure to the implants occurring for 10 days. The Low Dose animals received a single implant with an approximate area of 14.13 mm². The Middle

Dose animals received a single implant with an approximate area of 28.26 mm². The High Dose animals received two implants, each with an approximate area of 28.26 mm² for a total area of 56.52 mm². One group of mice served as Naive Control animals and did not undergo any surgical procedures. Another group of mice, serving as the Sham Control animals, underwent the same procedures as the High Dose animals except no implants were inserted. Other mice served as the Positive Control animals and received an appropriate known immunotoxic compound.

Serum Collection: On the 11th day after implantation in the earlier study, animals were bled prior to sacrifice. The blood was allowed to clot for a period of 1.5 to 2 hours. Blood was centrifuged for 10 minutes at 2000 rpm at a temperature set between 2°C and 8°C. For each blood sample, serum removed without red blood cell contamination was placed in a collecting vial that sealed tightly. All vials were stored in a -70°C freezer at [REDACTED] until shipped frozen to [REDACTED]

[REDACTED] Serum specimens remained frozen until thawed for tissue autoantibody evaluation.

Indirect Immunofluorescence Assay: Each serum specimen was diluted 1:10 with phosphate buffered saline (PBS), pH 7.4. Slides of the appropriate tissue substrate were covered by each serum dilution and incubated for 30 minutes at room temperature in a humid chamber. Tissue substrates consisted of frozen sections of tissue, cut at four microns thick and air dried, except for mouse thyroglobulin which had to be fixed since it is soluble. Mouse tissues were used for all the substrates.

Target Antigen	Mouse Tissue Used as Substrate
Nuclear Antigens	Liver
Parietal Cells	Stomach Mucosa
Smooth Muscle	Stomach Muscularis
Mitochondria	Kidney
Cardiac Striated Muscle	Heart
Skeletal Muscle	Skeletal Muscle
Pancreas Islet	Pancreas
Adrenal	Adrenal Gland
Thyroglobulin	Thyroid Gland

The tissues were then incubated with an anti-mouse immunoglobulin (IgG,A,M) that had been conjugated with a fluorescein isothiocyanate (FITC), to which a rhodamine-B counterstain was added. The slides

were examined with a fluorescence microscope equipped with filters for FITC. Positive fluorescence was seen as a bright green color against a yellow/orange background due to counterstain.

Positive and negative controls were included as well as a FITC-conjugate control. The positive control serum consisted of a pool of sera from MLR/lpr mice that previously showed a high titer of antinuclear antibody. The antinuclear antibodies present in the positive control MLR/lpr mouse serum reacts with cell nuclei in all evaluated tissues. The negative control serum was from a normal BALB/c mouse which previously tested negative for autoantibodies. Slides were examined blindly; positive fluorescence was graded according to the following scale:

<u>Intensity Scale</u>	<u>Amount of Fluorescence</u>
negative (-)	No Fluorescence
0.5 (or +/-)	Suspicious
1	Weak
2	Medium
3	Strong
4	Very Strong

Serum Evaluation: Serum specimens with a score of "1" (weak) or greater were titrated. Serial two-fold dilutions were made starting from the initial dilution of 1:10 and increasing to 1:20, 1:40 and 1:80. The immunofluorescence and titer of mouse autoantibodies to the target antigens were determined.

Cytokine Assays: Serum samples were also analyzed for four cytokines: interleukin-1-alpha (IL-1-a), interleukin-2 (IL-2), interferon-gamma (IFN-g), and tumor necrosis factor-alpha (TNF-a). The procedures used were per the cytokine kit manufacturer's instructions and utilized serum diluted 1:4. Due to the limited amount of serum, only a single data point was determined for each serum sample for each cytokine.

Results from the testing indicated that no significant differences were found for the serum autoantibody levels among the groups of this experiment. The few detectable autoantibody values could be accounted for by random variation. As seen in human studies, occasional autoantibody responses are present within the mouse population. Other than IFN-gamma, serum cytokine levels were not detected. No significant differences were found for the serum IFN-gamma levels among the treatment groups of this experiment.

ADJUVANT EFFECT OF SILICONE ELASTOMER PARTICLES IN RATS

In a past study¹ Naim et al. have reported that subcutaneous implantation of silicone gel material acts as an immunological adjuvant in response to bovine serum albumin. This raises the question as to whether under similar experimental conditions silicone elastomers, a major material component of Mentor Gel-filled Mammary Prostheses, also have adjuvant potential. To address this question and better understand the gel results, Noel Rose, M.D., Ph.D., The Johns Hopkins University, School of Hygiene and Public Health, Department of Molecular Microbiology & Immunology performed the study entitled "Adjuvant Effect of Silicone Elastomer Particles In Rats" (see the Biological Testing Appendices for the full report).

Study Objective: The primary purpose of this study was to (1) attempt to replicate the test system and silicone gel observations of Naim et al.¹, and (2) to investigate the adjuvancy potential of silicone oil and silicone elastomer particles in this same animal (rat) model system. Further, it was the objective of this study to determine and correlate the inflammatory potential of these articles, as determined by histopathologic examination, with the adjuvancy potential, if any, of the silicone gel and elastomers used in the manufacture of Mentor's Gel-filled Mammary Prostheses.

Study Design:

Test System: Sixty male Harlan Sprague-Dawley rats, approximately 250 grams each, after one week of quarantine, were used in this study. Animals found dead were immediately necropsied and the cause of death determined and tissues collected and fixed in 10% neutral buffered formalin for future histopathologic evaluation. At the end of the study, surviving rats were sacrificed and the left and right caudal thigh muscle and in some cases popliteal lymph node were fixed in 10% neutral buffered formalin for future histopathologic evaluation.

Study Duration: The appropriate test article was administered to each rat on day 1 of the study. Rats were sacrificed on day 55 with the exception of one group (Group G described below) which were sacrificed on day 35 of the study.

Experimental Groups (with ten rats per group):

- | | |
|---------|--|
| Group A | Incomplete Freund's Adjuvant (IFA) with Bovine Serum Albumin (BSA) |
| Group B | Silicone oil with BSA |

¹ Naim et al, Immunological Investigations 22 (2): 151-161, 1993

Group C	IFA/silicone oil with BSA
Group D	50% Silicone gel/50% silicone oil with BSA
Group E	Silicone oil/~ 1000 μ silicone elastomer particles with BSA
Group F	Silicone oil/~ 500 μ silicone elastomer particles with BSA
Group G	Saline with BSA

Test Article Preparation:

1. Silicone Elastomer Particles -
Siltex® Saline Mammary Prostheses with Mentor Leaf Valves were pulverized using a Wylie Mill and liquid nitrogen. Two groups of particles, one, ~ 500 μ in size, and the other ~ 1000 μ in size were prepared. **(Note - the textured surface described above was made from [REDACTED] Substantially equivalent [REDACTED] elastomer materials made by [REDACTED] [REDACTED] are currently being used to texture the surface of Low Bleed Gel-filled shells. For this reason, the adjuvancy data obtained on Siltex Saline-filled shells has relevancy to Siltex textured Gel-filled Low Bleed shells.)**
2. IFA and BSA -
An appropriate volume of IFA was mixed with an equal volume of BSA stock solution (0.34 mg/mL BSA prepared in PBS, pH 7.4, and sterilized by filtration). An emulsion was produced by trituration between two connected 10cc borosilicate glass syringes.
3. Silicone oil with BSA -
An appropriate volume of silicone oil (dimethylpolysiloxane, 20 cs) was mixed with an equal volume of BSA stock solution. Generation of an emulsion was attempted by trituration as discussed above.
4. IFA/ silicone oil with BSA -
An equal volume of IFA and silicone oil was mixed. Then an equal volume of BSA stock solution was added.

Generation of an emulsion was attempted by trituration as discussed above.

5. 50% Silicone gel/50% silicone oil with BSA -
An appropriate amount of silicone gel [REDACTED] obtained from a sterile Mentor Low Bleed Gel-filled Mammary Prosthesis) was placed in a pre-weighed container. The fluidity of the silicone gel was enhanced by homogenization using an Omni homogenizer fitted with a 7mM generator probe. After 6-8 cycles of homogenization, an equal volume of silicone oil (dimethylpolysiloxane, 20cs) was added. The silicone gel/silicone oil mixture was rehomogenized with an additional 6-8 cycles. Then an equal volume of BSA stock solution was added. Generation of an emulsion was attempted by trituration as discussed above. (Note - [REDACTED] gel has been replaced by a substantially equivalent [REDACTED] As a result, data from this testing is applicable to Mentor's current product.)
6. Silicone oil/~ 1000 μ silicone elastomer particles with BSA -
An appropriate volume of silicone oil was weighed and an equivalent weight of ~1000 μ silicone elastomer particles measured. Then BSA, at two times the volume of silicone oil was added to the silicone oil and made as described above. The emulsion was delivered into the injection site followed by the addition of the elastomer particles.
7. Silicone oil/~ 500 μ silicone elastomer particles with BSA -
An appropriate volume of silicone oil was weighed and an equivalent weight of ~500 μ silicone elastomer particles measured. Then BSA, at two times the volume of silicone oil, was added to the silicone oil and made as described above. The emulsion was delivered into the injection site followed by the addition of the elastomer particles.
8. Saline with BSA -
Equal volumes of normal saline (0.9%) and BSA stock solution were mixed and subsequently injected into the left caudal thigh muscle. Group G animals (saline with

BSA) were not included in the statistical analyses of anti-BSA antibodies or animal weights.

Dose Administration and Route of Administration: After anesthetization by inhalation of methoxyflurane, each animal was shaved in the left thigh muscle area, and a small incision was made on the skin followed by small incision in the muscle fascia. Using a 1 mL sterile glass pipette, 0.3 mL of the appropriate test article was delivered into the muscle. Animals receiving elastomer particles had the particles placed using a glass rod into the incision site subsequent to the infusion of the emulsion. The skin incision was closed using 2-3 stainless steel autoclips.

Blood Collection: Prior to test article administration, a control blood sample was obtained from each rat. After test article administration, blood samples were collected biweekly from each rat by cardiac puncture. Approximately 1 mL of blood was withdrawn slowly. The blood was allowed to clot at 4°C, sera collected, and stored at -70°C until used.

Animal Observation and Weight: Rats were observed daily to check their health status. Abnormal observations were documented. Rat body weights were determined upon receipt, at randomization, weekly during the study, and at sacrifice. Appropriate statistical analysis was performed to determine if there are statistically significant differences among the test groups in mean rat weight and weight change.

Histology: At the time of sacrifice, tissues at the implantation site (left caudal thigh) and the control site (right caudal thigh) were removed from each study animal, placed into separate vials containing 10% formalin, paraffin embedded, sectioned, stained with hematoxylin and eosin for light microscopic examination, and evaluated for histopathologic lesions. Animals were ranked according to the following classification scheme and the extent of the local inflammatory infiltrate was compared to the level of antiBSA antibody.

Histopathologic Classification Criteria

CLASSIFICATION	DESCRIPTION
MILD	<ul style="list-style-type: none"> · Absence of well formed granulomas · Minor inflammatory infiltrate (lymphocytes, plasma cells, neutrophils, eosinophils) · Vacuoles of varying size · Affects < 5% of tissue section
MODERATE	<ul style="list-style-type: none"> · Presence of small aggregates of macrophages · Small number of multinucleated giant cells · Moderate inflammatory infiltrate with or without lymphoid aggregates · Minor extension to underlying muscle · Affects 5-10% of tissue section
SEVERE	<ul style="list-style-type: none"> · Presence of well formed granulomas · Moderate to large numbers of multinucleated giant cells · Dense inflammatory infiltrate with or without lymphoid aggregates · Major extension to underlying muscle · Affects > 10% of tissue section

Serological Studies: Serum samples were thawed and Anti-BSA IgG titers were determined by enzyme-linked immunosorbent assay (ELISA) -

Wells of 96 well microtiter plates were coated with 50 µL of BSA solution (10 µL/mL) in carbonate buffer (pH 9.6), sealed in plastic wrap, and stored at 4°C.

Wells of the microtiter plate were washed with 0.05% Tween-20 in PBS (Wash solution)

Plates were blocked by adding 100 µL of wash solution to each well for 30 minutes at RTC (room temperature)

Each serum sample was diluted 1/250 with wash solution

50 µL of each sample was added to designated wells and tested in triplicate

Plates were incubated for 2 hours at RTC, then washed three times with wash solution

Alkaline phosphatase conjugated affinity pure goat anti-rat IgG, 50 µL, diluted 1/10,000 in wash solution was added to each well for 1 hour

Plates were washed 3 x with wash buffer, then 100 μ L of alkaline phosphatase substrate solution (1 mg/mL in diethanolamine-HCl buffer, pH 9.8) was added and the plates incubated in the dark.

The reactions were monitored kinetically at 10, 20, and 30 minutes after substrate addition with a microtiter plate reader (Dynateck MR600)

Data was collected using readings at the 30 minute interval.

Antibody response was computed by adjusting optical density readings using the following adjustment formula to compensate for interplate variation:

$$100/(\text{Positive control} - \text{Negative control}) = \text{X/O.D. reading}$$

Statistical tests were employed to determine differences among animal groups.

Results and Conclusions:

The silicone gel/silicone oil combination (group D) acts as a potent adjuvant with even greater adjuvant properties than IFA alone (group A); however, IFA mixed with silicone oil (group C) does not appear to have as great adjuvant properties as IFA alone (group A).

Groups B (silicone oil), E (silicone oil/~ 1000 μ silicone elastomer particles), and F (silicone oil/~ 500 μ silicone elastomer particles) had slightly elevated levels of anti-BSA antibodies on day 24 which dropped to zero on day 28.

Group G (saline with BSA) showed a similar pattern, i.e., slight expression of anti-BSA antibodies at day 14 up to the final days of the study.

Taking into consideration the slight adjuvant effect of BSA, the results of this study are consistent with the premise that particles derived from Siltex Saline-filled Mammary Prostheses with Mentor Leaf Valves (consisting of [REDACTED]) have no apparent adjuvant effect. Also, significant adjuvancy was not observed for silicone oil alone. In contrast, silicone gel was shown to have an adjuvancy potential in this test system. Thus, the physiochemical form (gel, elastomer, or fluid state) in which polydimethylsiloxane is presented to the immune system plays a role in

the degree of adjuvant response. Elastomer particles were without adjuvant effect.

Histopathological findings:

In all study groups, tissue sections of the right caudal thigh (control tissue) had no evidence of significant inflammation.

Group A (Incomplete Freund's Adjuvant/BSA) animals typically showed a moderate to severe inflammatory infiltrate, vacuolation, granulomas surrounded by dense infiltrate of inflammatory cells (lymphocytes, plasma cells, neutrophils, occasional eosinophils, and multinucleated giant cells).

Group B (silicone oil with BSA) There was little evidence of lymphocytic infiltrate. Only occasional aggregates of macrophages and isolated multinucleated giant cells were observed.

Group C (Incomplete Freund's Adjuvant/silicone oil/BSA) animals ranged from mild to extensive inflammatory lesions similar to Group A.

Group D (50% silicone gel/50% silicone oil/BSA) animals have abundant foamy macrophages, a dense well-organized lymphoplasmic inflammatory infiltrate, and often extend into the underlying muscle bundles.

Group E (silicone oil/~ 1000 μ silicone elastomer particles/BSA) a local, mild inflammatory response was observed.

Group F (silicone oil/~ 500 μ silicone elastomer particles/BSA) a local inflammatory response slightly greater than Group E was observed.

Good adjuvants usually elicit an intense local inflammatory response. Silicone elastomers, under the conditions of this study, do elicit a mild to moderate inflammatory response, but fail to enhance production of anti-BSA antibodies. Silicone elastomers do not appear to have adjuvant properties. In contrast to elastomers, silicone gel, under the conditions of this study, does appear to have adjuvant properties.

Since the completion of Prof. Rose's study above, Klykken and White² have subsequently shown that in the absence of premixing the antigen with the silicone test material (as was performed in the Rose study), there does not appear to be any silicone induced adjuvant response. Finally, Kervliet (1998) in the National Science Panel Report to the Honorable Sam Pointer Jr. has reviewed this data and other data related to the adjuvancy of silicone gel.³ "The ability of silicone to act as an adjuvant has received a lot of attention. Even though some silicone gels and fluids have been shown to possess adjuvant activity when antigen is emulsified with the silicone prior to immunization, this capability has little bearing on the issue of silicone- induced autoimmune disease. It most likely reflects a depot effect of the non-degradable silicone. There are no convincing data that show silicone acts like an adjuvant when it is present at a site distant from the antigen injection, and there is no biologically plausible mechanism for antigen emulsification to take place in the body."

REPRODUCTION/TERATOGENICITY BIOASSAY

Mentor Low Bleed Shell Testing

The available studies of potential developmental and reproductive toxicity associated with exposure to various forms of the polydimethylsiloxanes (discussed in the literature review section) have indicated that these compounds lack teratogenic activity and are not reproductive toxicants. Additionally, Mentor designed a study to assess the teratogenic and reproductive potential associated with implantation of Mentor's Gel-filled Mammary Prosthesis shell. The study was performed by UBTL, Inc. in accordance to Good Laboratory Practices as set forth by the FDA, 21 CFR 58. The final report can be found in **MENTOR'S SALINE-FILLED MAMMARY PMA (#P940039/A7)** Biological Testing Appendices (submitted May 4, 1995), Vol. V.C.3, No. 22 to 23 of 31 books, pp 7183 - 7818.

Test Article Identification and Dosage: Mentor manufactures many different types of mammary prostheses, both saline-filled and silicone gel-filled. In order to simplify the manufacturing operation, many of the materials and components are used in similar ways for several types of these devices (e.g., the same textured surface layer is used for Siltex Saline-filled and Siltex Gel-filled shells). As a result, in order to limit the complexity of this biological test, Mentor chose not to test a sample of every type of device made, but

² Klykken P., White, K., " The Adjuvancy of Silicones: Dependency on Compartmentalization" in Immunology of Silicones, Potter and Rose eds., Springer-Verlag, New York, 1996.

³ Kervliet, N., Review of Animal Studies Relevant to Silicone Toxicity. In: Silicone Breast Implants in Relation to connective Tissue Diseases and Immunologic Dysfunction. National Science Panel, 1998 Nov. 17.

instead chose enough samples from selected finished product mammary prostheses to contain all the materials used in all of Mentor's mammary implant lines. The similarities in the manufacturing conditions for all mammary product lines made this a justifiable alternative. [Note - this report contains testing performed on a Low Bleed Gel-filled shell made using [REDACTED] silicone dispersion materials. Since the time that this test was performed, Mentor has changed to substantially equivalent silicone materials for this component. The gel-filled mammary shell (made using [REDACTED] and some of the device components made from silicone elastomer [REDACTED] are now made using materials from [REDACTED]. Device component silicone elastomer [REDACTED] may also continue to be purchased [REDACTED] information as well as finished device physical and chemical extract data supplied by Mentor demonstrated that all of the replacement materials are substantially equivalent to the original [REDACTED] materials; therefore, Mentor believes that this biological testing is directly applicable to the products and materials in this PMA.]

The test articles consisted of patched and/or valved silicone elastomer prosthetic shells pulverized, suspended in sesame oil, and subcutaneously implanted in rats. The animal groups were:

Sham Control (sesame oil)	0 mg
Low Bleed Shell with Patch	200 mg
Siltex (textured) Low Bleed Shell with Outer Becker Valve	450 mg
RTV Smooth Shell with Diaphragm Valve	280 mg
RTV Textured Shell with Mentor Leaf Valve	430 mg

The dose of test article used in this study was based upon the implantation of an amount (mg) of each test article in female Sprague-Dawley rats (estimated average weight of 333 gm) equivalent on a relative body weight basis to the weight of two 800 ml sized patched and/or valved silicone elastomer mammary prosthetic shells in a 60 kg (average weight) human female. In order to exaggerate the dose of potentially extractable materials, the elastomeric test material was pulverized prior to implantation, thus vastly increasing the exposed surface area. The weights of the four different shell configurations differed due to the presence of the textured surface and the construction of the patches and valves. Based on the volumes of sesame oil added, the concentration of each test article was adjusted, allowing each animal to receive the required dose of test article in 1.2 ml of the sesame oil suspension.

Evaluation of the reproductive and teratogenic potential specific to the various shell configurations of Gel-filled Mammary Prostheses is based upon the results associated with the following test articles: Low Bleed Shell with Patch and Siltex (Textured) Low Bleed Shell with Outer Becker Valve, as compared

to the Sham Control. The Low Bleed Shell with Patch primarily tests the smooth Gel-filled shell and the Siltex (Textured) Shell with Outer Becker Valve primarily tests the [REDACTED] silicone textured surface and patch along with the smooth gel-filled shell. These two samples encompass the components and materials found in all of the types of Smooth and Siltex Gel-Filled Mammary Prostheses contained in this PMA.

Test Article Preparation: In order to generate particulate samples for implantation, each test article (consisting of patched and/or valved silicone elastomer mammary prosthesis shell) was frozen in liquid nitrogen and placed in a Waring blender containing liquid nitrogen. The blender contents were pulverized and sieved through a No. 18 U.S. Standard Sieve Series (1 mm). Each test article was collected, weighed into separate tared beakers, and suspended in sesame oil. Each test article in sesame oil suspension was steam sterilized prior to implantation.

A volume of sesame oil sufficient for the implantation of 1.2 ml per control animal was placed in beakers and steam sterilized prior to implantation.

Test articles were prepared on two occasions; first for implantation of the F0 generation female rats and later for implantation of the F1 generation female rats.

Test System: Male Sprague-Dawley rats weighing approximately 180 to 223 grams, and female Sprague-Dawley rats weighing approximately 149 to 178 grams were obtained from Charles River Laboratories, Wilmington, MA. All animals were approximately 50 days old at the time of receipt. Male rats were used for mating purposes only and were not implanted with test article.

Surgical Procedure: All surgical procedures were performed aseptically. After the animals were appropriately prepared, two small incisions, one on each side of the spinal column were made in the skin in the sacrolumbar area of the back. At each incision site, a subcutaneous tract 5 to 6 cm in length and parallel to the spinal column, was formed anteriorly with blunt dissection.

For each surgical tract in the four test article group animals, 0.6 ml of the designated test article suspended in sesame oil was taken up in a sterile 1 ml syringe. The syringe was inserted into a surgical tract; the syringe contents injected into the tract. The empty syringe was removed; the incision site closed. Each animal received 1.2 ml of sesame oil containing the appropriate dose of test article (two 0.6 ml volumes). The Sham Control rats received 0.6 ml of sesame oil per surgical tract, resulting in each rat receiving 1.2 ml of the sesame oil.

Female rats of the F0 generation were implanted when approximately 10 weeks old. Female rats of the F1 generation were implanted when

approximately 6 - 10 weeks old, following the same procedure as performed on the F0 generation. As stated previously, male rats were not implanted with the test articles.

Study Design and Parameters: Thirty five female adult Sprague-Dawley rats, designated as the F0 generation, were assigned to each of the four test article groups. Animals were implanted with the appropriate test article. An additional thirty-five female adult Sprague-Dawley rats were assigned to the Sham Control group and were implanted with sesame oil.

Approximately seven weeks after surgery, the F0 female (implanted) and male rats were mated to produce the F1 generation rats. Cohabitation for purposes of mating continued until all female rats in a group had been mated or three weeks of mating was completed.

As the gestation period ended, the female rats were observed for evidence of delivery. After parturition, pups were observed twice daily for viability. On Days 0, 1, 4, 7, 14, and 21 of lactation, the following observations were made: the number of live and dead pups, weight and sex of each pup, and external appearance of each pup. Pups found dead were necropsied. On Day 4 of lactation, litters consisting of more than 10 pups were reduced (culled) to 10 pups. Culled pups were necropsied. On day 21 of lactation, litters were culled to 4 pups. At the time of weaning, 35 female F1 generation rats and 35 male F1 generation rats from each groups were selected to continue the F1 generation to maturity. The non-selected F1 generation rats and the F0 generation rats were sacrificed and necropsied.

The selected F1 generation female rats were surgically implanted with the same test article as their respective dams. Following a recovery period of approximately six weeks, the F1 generation female (implanted) and male rats were mated to produce the F2a generation offspring. On Day 4 of lactation, litters consisting of more than 10 pups were culled to 10 pups. The culled pups were euthanized and necropsied. On Day 21 of lactation the F2a generation offspring were sacrificed and necropsied.

After a two week rest period, the F1 generation female and male rats were remated for the teratology phase of the study (F2b fetuses). Mated F1 generation female rats were sacrificed and necropsied on Day 20 of gestation. The uterus from each sacrificed animal was evaluated. The fetuses were examined and divided into two groups. One group of fetuses was preserved for visceral evaluation. The other group of fetuses was preserved for skeletal evaluation. The male F1 generation rats were sacrificed and necropsied. The testes and epididymides were weighed and histopathologically evaluated.

Results: Compared to the sesame oil control, implantation of the pulverized patched and/or valved silicone elastomer mammary shell test articles in two

generations of female Sprague-Dawley rats did not affect mating, gestation or lactation in the female rats. Implantation of the test material, relative to the sesame oil, did not affect offspring parameters (teratology phase) including: numbers, sex, weight, internal and external appearance, and survival. Furthermore, the test material did not affect fetal parameters including: numbers, external appearance, and the type and incidence of visceral and skeletal alterations and variations as compared to the control.

A foreign body response at the implant sites was elicited in both the F0 and F1 generation female rats subcutaneously implanted with the test articles. The response was similar for the four test groups and was similar for the rats in both generations.

The reproductive performance of all groups (including controls) was lower than expected especially during the second F1 generation mating (to produce the F2b generation) as evidenced by the fertility index and time to mate. To further investigate this finding, the epididymides and testes of the F1 generation male rats were weighed and evaluated histopathologically. Although there were a few, scattered incidences of low testicular weight and/or histological evidence of aspermia, an underlying cause of the reduced reproductive performance was not determined. Other factors that may have influenced the reduced reproductive performance include obesity and advancing age. However, since the reproductive performance was reduced for all groups, including the Sham Controls, this finding is not considered test article related.

The results of this study indicate that, compared to the controls, the pulverized patched and/or valved silicone elastomer mammary prosthetic shell test samples do not cause reproductive or teratologic effects when implanted subcutaneously in female rats in two consecutive generations. These results are consistent with the available published studies of animals exposed to various forms of the polydimethylsiloxanes, which indicate that these compounds lack teratogenic activity or reproductive toxicity.

Silicone Gel Testing

Prior to the use of [REDACTED] silicone gel, Mentor used [REDACTED] silicone gel [REDACTED] and [REDACTED] for gel-filled mammary prostheses. [REDACTED] these earlier silicone gels had [REDACTED] [REDACTED] the cured gels Mentor used. (Dow Corning has since made much, if not all, of this information publicly available.) As part of the qualification of [REDACTED] [REDACTED] substantial equivalence to Dow Corning gel was demonstrated

by raw materials testing in the MAF as well as Mentor's own physical and chemical extract testing on finished devices. As a part of Mentor's [REDACTED] qualification, evidence of substantial equivalence between the [REDACTED] [REDACTED] silicone gel was provided using the same types of analyses. For these reasons, Mentor believes that the reproduction/teratology data contained in these [REDACTED] provides the necessary information to demonstrate the reproductive and teratological safety of these silicone gels, and because of substantial equivalence, the [REDACTED]

Please refer to [REDACTED] for teratology testing on their [REDACTED]. Dow Corning published the results of their reproduction and teratology study.⁴ A copy of the article is included in the appendices of this section and the results are summarized below.

Dow Corning: Reproductive and Developmental Toxicity Studies of Silicone Gel Q7-2159A in Rats and Rabbits⁴

Studies reported here assessed the potential adverse effects of silicone gel, Dow Corning Q7-2159A, on general reproduction and fetal development in male and female Charles River CD rats and New Zealand white rabbits. Two control and three treatment groups of 30 male and 30 female rats and 25 female rabbits per group were used in the one-generation reproduction and developmental toxicity studies, respectively. The silicone gel was implanted subcutaneously in two flank sites at dosage levels of 3, 10, and 30 ml/kg. The highest dose was selected on the basis of likely human body burden. Control groups received either sterile saline or carboxymethylcellulose solution in two flank implantation sites. The control and test articles were implanted in male and female rats 61 and 47 days, respectively, prior to mating (in the rat reproduction study) and approximately 42 days prior to insemination of female rabbits (in the rabbit developmental toxicity study). There were no treatment-related effects on F₀ parental general conditions and reproductive performance, F₁ neonatal viability, or growth in the rat reproduction study. No maternal nor developmental effects, including teratogenicity, were observed in rabbits in the groups implanted with Q7-2159A gel in the developmental toxicity study.

⁴ Siddiqui, W.H., Schardein, J.L., Cassidy, S.L., Meeks, R.G., *Fundamental and Applied Toxicology* 23: 370 - 376, 1994

TWO YEAR CHRONIC TOXICITY/CARCINOGENICITY BIOASSAY

Mentor Gel-filled Mammary Shell Testing

A study was designed to assess the chronic toxicity and carcinogenicity potential of Mentor's Smooth and Siltex Gel-filled Mammary Prostheses shells. The study was performed by UBTL, Inc. in accordance with Good Laboratory Practices as set forth by the FDA, 21 CFR 58. The full length 3, 12, and 24 month reports are located in **MENTOR'S SALINE-FILLED MAMMARY PMA (#P940039/A7)** Biological Testing Appendices (submitted May 4, 1995), Vol. V.C.3, No. 10 to 21 of 31 books, pp. 3162 - 7182. Based on the final two year study report, the materials associated with these gel-filled mammary prostheses shells did not cause chronic toxicity and only produced implant-site-related sarcomas representative of the typical solid-state tumorigenesis observed in rodents following implantation of a wide range of foreign materials. As discussed in the literature review section of this submission (Section IV) under Long-term (Carcinogenicity) Studies, the lack of relevance of solid-state tumorigenesis to human risk has been previously recognized.

Test Article Identification and Dosage: Mentor manufactures many different types of mammary prostheses, both saline-filled and silicone gel-filled. The manufacturing operation for these devices is streamlined such that many of the materials and components are used in similar ways for several types of these devices (e.g., the same textured surface layer is applied to Siltex Saline-filled and Siltex Gel-filled shells). As a result, test samples for this two-year biological test were selected from finished product mammary prostheses to contain all the materials used in all of Mentor's mammary implant lines. The similarities in the manufacturing conditions for all mammary product lines are consistent with this rationale.

The test articles consisted of patched and/or valved silicone elastomer prosthetic shells pulverized, suspended in sesame oil, and subcutaneously implanted in rats. The animal groups were:

Sham Control (sesame oil)	0 mg
Low Bleed Shell with Patch	200 mg
Siltex (textured) Low Bleed Shell with Outer Becker Valve	450 mg
Smooth RTV Shell with Diaphragm Valve	280 mg
Textured RTV Shell with Mentor Leaf Valve	430 mg

The dose of test article used in this study was based upon the implantation of an amount (mg) of each test article in female Fisher 344 rats (estimated average weight of 333 gm) equivalent on a relative body weight basis to the weight of two 800 ml sized patched and/or valved silicone elastomer mammary

prosthetic shells in a 60 kg (average weight) human female. In order to exaggerate the dose of potentially extractable materials, the elastomeric test material was pulverized prior to implantation, thus vastly increasing the exposed surface area. The weights of the four different shell configurations differed due to the presence of the textured surface and the construction of the patches and valves. Based on the volume of sesame oil added, the concentration of each test article was adjusted, allowing each animal to receive the required dose of test article in 1.2 ml of the sesame oil suspension.

Evaluation of the chronic toxicity and carcinogenic potential specific to the various configurations of Gel-filled Mammary Prostheses is based upon the results associated with the following test articles: Low Bleed Shell with Patch and Siltex (Textured) Low Bleed Shell with Outer Becker Valve, as compared to the Sham Control. The Low Bleed Shell with Patch primarily tests the smooth Gel-filled shell and the Siltex (Textured) Shell with Outer Becker Valve primarily tests the [REDACTED] textured surface and patch along with the smooth gel-filled shell. These two samples encompass the components and materials found in all of the types of Smooth and Siltex Gel-Filled Mammary Prostheses shells. In addition, because of the equivalence of today's materials with those used in these tests, the results summarized below are directly applicable to the devices and materials in this PMA

Test Article Preparation: In order to generate particulate samples for implantation, each test article (consisting of a patched and/or valved silicone elastomer mammary prosthesis shell) was frozen in liquid nitrogen and placed in a Waring blender containing liquid nitrogen. The blender contents were pulverized and sieved through a No. 18 U.S. Standard Sieve Series (1 mm). Each test article was collected, weighed into separate tared beakers, and suspended in sesame oil. Each test article in sesame oil suspension was steam sterilized prior to implantation.

A volume of sesame oil sufficient for the implantation of 1.2 ml per control animal was also placed in beakers and steam sterilized prior to implantation.

Test System: Female Fischer 344 rats approximately 8 to 10 weeks of age and weighing approximately 110 to 160 grams at the time of surgical implantation, were obtained from Charles River Laboratories, Raleigh, NC.

Surgical Procedure: All surgical procedures were performed aseptically. After the animals were appropriately prepared, two small incisions, one on each side of the spinal column were made in the skin in the sacrolumbar area of the back. At each incision site, a subcutaneous tract 5 to 6 cm in length and parallel to the spinal column, was formed anteriorly with blunt dissection.

For each surgical tract in the four test article group animals, 0.6 ml of the designated test article suspended in sesame oil was taken up in a sterile 1 ml

syringe. The syringe was inserted into a surgical tract; the syringe contents injected into the tract. The empty syringe was removed; the incision site closed. Each animal received 1.2 ml of sesame oil containing the appropriate dose of test article (two 0.6 ml volumes). The Sham Control rats received 0.6 ml of sesame oil per surgical tract, resulting in each rat receiving 1.2 ml of the sesame oil.

Study Design and Parameters: Eighty Fischer 344 rats were assigned to each of the five study groups. Each study group was comprised of the main group (sixty rats, followed for lifetime) and the satellite group (twenty rats). Ten satellite rats from each study group were sacrificed after 3 months implantation; the remaining satellite rats were sacrificed after 12 months implantation.

Throughout the in-life phase, the implanted animals were observed twice daily for viability, weekly for general health, and measured regularly for body weights. Clinical chemistry and hematology data was collected prior to implantation and, again, prior to scheduled sacrifice. Gross necropsy and organ weight determinations and histopathologic evaluations on selected organs were performed on sacrificed animals.

Results: At three, twelve, and twenty four months after the surgical implantation of the test articles, no biologically significant changes that could be attributed to the subcutaneous implantation of test articles were noted for: rat survival, body weight, clinical chemistry parameters, hematology parameters, and organ weights. Spontaneous lesions and incidental findings involving the heart, kidneys, lung, liver, spleen, and lymph nodes common to female Fischer 344 rats were observed in all groups and were not considered test article related.

Changes attributable to treatment were those associated with the subcutaneous implantation of the pulverized test articles as noted during general health checks. During the first 3 months the incidence of alopecia, erythema, ulceration, swelling, open wounds and scabbing at the implant sites observed in the test article groups was generally increased as compared to the Sham Control group. These findings tended to diminish as the local tissue response began to resolve. During the second year, the development of palpable masses in the proximity of the implantation sites were observed in all of the implanted groups, even in the Sham Control group. The number of animals with palpable implant site masses increased with increasing time of exposure.

Histopathological evaluation of the implant sites 3 and 12 months after implantation revealed changes in the tissue consistent with a chronic foreign body response including: fibrous encapsulation of the test article, aggregation of macrophages, multinucleated foreign body giant cells, and multiloculated

cyst-like areas. Generally, the incidence and/or severity of these findings were less in the Sham Control group.

The 12 month histopathological evaluation also indicated that fibrosarcomas were associated with the implant sites of four animals: three Siltex Shell with Outer Becker Valve group animals and one RTV Textured Shell with Mentor Leaf Valve group animal. Other neoplasms observed among the animals included: mononuclear cell leukemia in one RTV Smooth Shell with Diaphragm Valve group animal and endometrial polyps in two animals, one in the Siltex Shell with Outer Becker Valve group and one in the RTV Textured Shell with Mentor Leaf Valve group.

Two years after the surgical implantation of the test articles, no biologically significant changes in survival, body weight, clinical chemistry, hematology parameters, or in organ weights were noted that were attributed to the subcutaneous implantation of the test articles.

The non-neoplastic and neoplastic lesions observed at the implant sites of the test articles were similar to those seen during the first year of the study. They were characterized by multiloculated cyst-like areas surrounded by fibrous capsules, test article, macrophage aggregates, foamy macrophages, multinucleated foreign body giant cells, pigment containing macrocytes, and focal or multifocal mineralization. Implant related fibrosarcomas were noted in one animal of the Sham Control group, 18 animals of the smooth RTV Shell with diaphragm valve group, 26 Siltex (textured) Shell With Outer Becker Valve group, and 23 Textured (RTV) with Mentor Leaf Valve. Variable amounts of collagen were observed in all of the fibrosarcomas.

It is important to note that although there was some variation in the numbers of fibrosarcomas among the implanted groups, the histological appearance of the fibrosarcomas was similar regardless of the chemical composition of the implanted silicone elastomer. Further, it is known that rodents are highly susceptible to the production of foreign body sarcomas following the subcutaneous implantation of a variety of "inert" materials. In fact, in this study a fibrosarcoma apparently arose from the fibrous scar tissue formed at the incision site of one of the Sham Control animals. The presence of fibrosarcomas at the implant sites represents a neoplastic response common to rodents following the subcutaneous implantation of a foreign body.

No other treatment-related histopathologic alterations, non-neoplastic or neoplastic, were observed in the lungs, liver, spleen, kidneys, heart, mammary glands, or in the axillary and cervical lymph nodes from implanted groups compared with the Sham Control group. The types of lesions observed in other organs and tissues were considered typical of female Fisher 344 rats of this age.

Conclusion: Under the conditions of this study, the subcutaneous implantation of the Mentor Gel-filled Mammary shell test articles produced no evidence of systemic toxicity as measured by survival (mortality) body weight, organ weight (absolute or relative to brain or body weight), hematology, and serum chemistry examinations. Changes attributable to the test articles were observed at the site of implantation. They included various degrees of inflammatory responses as evidenced by edema, scabbing, alopecia, erythema, ulceration, swelling, open wounds, wet red/black/brown material and scarring. Microscopically the changes were characterized by multiloculated cyst-like areas surrounded by fibrous capsules, the presence of test article, macrophage aggregates, foamy macrophages, multinucleated foreign body giant cells pigment containing macrocytes, and focal or multifocal mineralization. Carcinogenicity was limited to the development of implant site related sarcomas, primarily fibrosarcomas, and their subsequent effects. The presence of fibrosarcomas at the implant sites represents a neoplastic response commonly observed in rodents following the subcutaneous implantation of a broad range of foreign materials. As discussed in literature reviews on the potential carcinogenicity of silicone, it has been recognized that solid-state carcinogenesis observed in rodents is not considered relevant to human risk. Furthermore, strong epidemiological evidence from the two large studies involving women with mammary prostheses indicates that there is no increased risk of breast cancer associated with these devices.

Gel-filled Mammary Gel Testing

Since the 1980's, Mentor has utilized [REDACTED] silicone gel, first [REDACTED]

Each of the vendors submitted [REDACTED] included chronic toxicity/carcinogenicity testing on gel cured using conditions substantially equivalent to those that Mentor uses in production.

Both the [REDACTED] included side by side testing of their gel to [REDACTED] gel. In addition, Mentor performed finished device chemical extractables testing to show that sterile devices made from [REDACTED] were substantially equivalent to each other and to devices made with [REDACTED] gel. For these reasons, Mentor believes that the [REDACTED] chronic toxicity/carcinogenicity data provided in [REDACTED] which demonstrated their long term safety is directly applicable to the [REDACTED] gel currently in use by Mentor.

Please refer to [REDACTED] for their chronic toxicity/carcinogenicity testing results. [See Mentor's PMA #P910037/A35, dated September 20, 1996, Vol. 1, pg 5 for [REDACTED]

Dow Corning has publicly released the gel chronic toxicity/carcinogenicity data which it filed with FDA as part of its 1991 Gel-filled Mammary PMA. A copy of Dow Corning's summaries of their studies follows this page. More detailed reports of the studies can be found in the Appendices of this Biological Testing Section. In addition, one of the studies mentioned in Dow Corning's summary (Report Reference 154) was not completed at the time of their PMA submission; however, it was subsequently submitted to the USEPA and a copy of that report has also been provided in the Appendices of this PMA Biological Testing Section.

Mentor believes that all of the above mentioned chronic toxicity/carcinogenicity studies on silicone gels together demonstrate their lack of long-term systemic toxicity issues. As a result of the equivalence between the tested gels and [REDACTED] Mentor also believes that the data strongly supports a similar lack of chronic toxicity issues related to the use of the [REDACTED] gel.

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SILICONE GEL:

The silicone gels used in Dow Corning's mammary implants are designated Q7-2159A which is the cured product of Q7-2167 and Q7-2168 combined in a 3:1 ratio, and Q7-2151 INT, which is the cured product of Q7-2150 and Q7-2146, also combined in a 3:1 ratio. Q7-2167 and Q7-2168 are also sold by Dow Corning to other manufacturers of silicone gel mammary implants. Non-clinical safety studies are available for a number of silicone gel formulations identified by product number in addition to Q7-2159A and Q7-2151. All of these gels employ fundamentally the same chemistry and differ primarily with regard to the physical property - penetration. Studies of all these gels are included here for the purpose of providing a complete overview of the biological properties of methylsiloxane polymers in the gel form.

ACUTE TOXICITY:

1. **SYSTEMIC/EYE/SKIN -**

A single rabbit skin irritation test is available using gel X7-9172 (Report Reference 104). This gel formulation is not a skin irritant.

2. **IN VITRO CYTOTOXICITY -**

Tissue culture biocompatibility testing usually employed WI-38 with Chang liver cells also sometimes used. The tabulated results indicate that Q7-2159A and related silicone gel formulations are not cytopathic. The single exception is a specific lot of Q7-2159A which did exhibit cytotoxicity which proved to be caused by incomplete mixing of butyl carbitol acetate, the catalyst solvent (Report Reference 105).

TISSUE CELL CULTURE BIOCOMPATIBILITY*

Gel	Result
Q7-2159A	Cytotoxic
Q7-2159A	NCE#
X3-0885	NCE

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described for all the other silicone gels. At the subcutaneous sites the reaction was of the foreign body type except the gel masses underwent progressive trabeculation with fibrous connective tissue septa beginning between 2 and 4 weeks after gel implantation. Small numbers of macrophages and giant cells were seen throughout the 180 day period.

In summary, the ten chronic studies reviewed here indicate that silicone mammary gel Q7-2159A and a number of gel variations are all associated with the foreign body reaction characteristic of a broad range of persistent alloplastic materials. That is, an early acute inflammatory response is superseded by a chronic inflammatory phase (i.e., an infiltrate of predominantly macrophages) that transitions to fibroblastic activity and fibrous connective tissue encapsulation of the foreign body. A sparse dispersal of macrophages and giant cells may persist long-term although this is not characteristic of all implantation sites in all animals.

In addition, silicone gel instilled subcutaneously without a limiting envelope can undergo progressive trabeculation with connective tissue septa of the same composition as the primary connective tissue capsule. The sequence of cellular infiltration with connective tissue replacement occurs as seen with the primary encapsulation.

CHRONIC TOXICITY:

Several chronic studies of silicone gels are available (Report References 152-155).

1. LIFE-TIME RAT STUDY OF MDF-0193, Q7-2159A AND TWO SILICONE GELS OF INTERMEDIATE FORMULATION (Report Reference 152) -

Dow Corning initiated a 2-year rat study of subcutaneously implanted silicone gels in 1975 at Industrial Bio-Test. The silicone gels included MDF-0193 which is the original so-called "stiff gel" and 3 gels of lower consistency that are closely related to the presently used so-called "responsive gel", Q7-2159A. The end of the in-life phase of this study coincided with the legal difficulties experienced by Industrial Bio-Test. Thus, an incomplete final report was delivered to Dow Corning such that it was necessary to contract elsewhere for the histopathology evaluation. Histopathology was evaluated and reported by an independent pathologist who concluded that there was an excess incidence of malignant lymphoma and an excess

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incidence of sarcomas all of which were associated with the implantation site. At a later date, the entire study was re-evaluated by yet another pathologist with participation of a referee pathologist in resolving instances of doubt. It is this latter analysis that is summarized here.

This life-time rat study consisted of implanting subcutaneously one gm of gel/kg initial body weight in groups of 50 animals of each sex. There were 4 silicone gel formulations tested and compared to an untreated (housing) control and a surgical sham control.

The malignant lymphoma data are summarized in the table.

NUMBER WITH LYMPHOMA/NUMBER WITHOUT LYMPHOMA#

	Males	Females	Total
Untreated Control (UC) (Responsive Gel)	23/24	9/41	32/65
Sham Control (SC)	20/27	28/21 P=0 vs. UC*	48/48 P=0.061 vs. UC
TX-1208 (Q7-2159A)	17/32	19/28 P=0.015 vs. UC	36/60
TX-1209	16/32	31/17 P=0 vs. UC	47/49
TX-1210	30/10 P=0.002 vs. UC P=0 vs. SC	37/11 P=0 vs. UC P=0.037 vs. SC	76/21 P=0 vs. UC P=0 vs. SC
TX-1211 (MDF-0193) (Original Gel)	39/9 P=0.01 vs. UC P=0 vs. SC	40/10 P=0 vs. UC P=0.014 vs. SC	79/19 P=0 vs. UC P=0 vs. SC

The lesions enumerated were first diagnosed as malignant lymphoma. The re-evaluation diagnosed them as white cell infiltrations associated with an infective process.

• Chi² test.

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The study re-evaluation corrected the misdiagnosis of malignant lymphoma and also presented a diagnosis of sarcomas at the material implantation sites not noted by the original pathologist. These findings are summarized in the following table.

NUMBER WITH SITE SARCOMA/NUMBER WITHOUT SITE SARCOMA

	Males	Females	Total
Untreated Control (UC) (Responsive Gel)	---	---	---
Sham Control (SC)	0/47	0/49	0/96
TX-1208 (Q7-2159A)	7/42	2/45	9/87
TX-1209	1/47 P=0.029* vs. TX-1208	1/47	2/94 P=0.030 vs. Tx-1208
TX-1210	1/48	4/44	5/92
TX-1211 (MDF-0193 (Original Gel))	0/48	3/47	3/95

* Chi² test.

Neither the Untreated Control nor Sham Control groups had an incidence of implantation site sarcomas because no material was implanted in these groups. These were no notable differences in the incidence of sarcomas between the silicone gel groups. A majority of the sarcomas were fibrosarcomas. All other pathology was comparable across groups and concluded to be within the limits of expectation for rats of this age and strain.

2. LIFE-TIME RAT STUDY OF MDF-0193 AND Q7-2159A (Report Reference 153) -

Dow Corning initiated an in-house 2-year rat study of subcutaneously implanted silicone gels in 1985. The silicone gels included MDF-0193 which is the original so-called "stiff gel" and the presently used so-called "responsive gel", Q7-2159A. This study conformed to Good Laboratory Practices.

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This life-time rat study consisted of implanting subcutaneously 4.0 gm of gel/kg initial body weight in groups of 50 male rats and 2.5 gm/kg initial body weight in groups of 50 female rats with the objective of achieving a constant 10 gm/kg body burden in the fully mature animals.

There were 2 silicone gel formulations tested and compared to a surgical sham control.

No biologically significant treatment-related effects were observed with regard to clinical chemistry, hematology, urinalysis, food consumption, body weights or organ weights. Cumulative survival was depressed among males implanted with either Q7-2159A or MDF-0193 but not among females. The biologic relevance of this observation is unknown.

Implantation site-associated mesenchymal neoplasms (i.e., sarcomas) were seen in both silicone gel treatment groups as summarized in the following table.

INCIDENCE OF SARCOMAS AND FIBROMAS

	MALE			FEMALE		
	Sham	Q7-2159A	MDF-0193	Sham	Q7-2159A	MDF-0193
Fibro-sarcoma	0/50	12/50	11/50	0/50	8/50	10/50
Chondro-sarcoma	0/50	1/50	0/50	0/50	0/50	0/50
Sarcoma, Undiff.	0/50	0/50	0/50	0/50	2/50	2/50
Fibroma	0/50	0/50	0/50	0/50	1/50	0/50
TOTAL	0/50	13/50	11/50	0/50	11/50	12/50

The sarcomas were associated with the connective tissue capsule of the implanted materials and the majority were fibrosarcomas. There were no statistical differences in sarcoma incidence between silicone gel groups. A few metastatic sarcomas were

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detected in animals with a site sarcomas; no sarcomas remote from the implantation site were observed in animals that did not have a site sarcomas.

The incidence of mammary adenocarcinomas and fibroadenomas was not different between treatment groups. In the MDF-0193 male group only the incidence of hepatocellular adenoma was increased above the control group level at $p < 0.20$ but not at $P \leq 0.05$.

A number of non-neoplastic lesions occurred with statistical significance ($P \leq 0.05$) as summarized in the following list. These changes were judged by the pathologist to be stress related rather than treatment related.

NON-NEOPLASTIC EVENTS SIGNIFICANT AT $P \leq 0.05$ *

Males	Females
<u>Q7-2159A:</u>	<u>Q7-2159A:</u>
Decreased seminal vesicle secretion	Increased extramedullary spleen hematopoiesis
Increased stomach necrosis	Increased stomach necrosis
Increased thymic hemorrhage	
Increased pyelitis#	
Increased liver necrosis#	
<u>MDF-0193:</u>	<u>MDF-0193:</u>
Adrenal extramedullary hematopoiesis	Increased thymic involution
Increased thymic hemorrhage	Uterine cystic glands
Increased lung edema#	

* These changes were judged by the pathologist to be stress related rather than treatment related.

These events could not be attributed to stress and may be treatment related.

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In summary, the principal findings of this study are:

- A. The presence of implant site-associated sarcomas for both test materials in both sexes. This effect is consistent with tumorigenesis produced by solid-state tumorigenesis, also known as the Oppenheimer effect. This mechanism is generally thought to be epigenetic and applicable to persistent alloplastic materials.
- B. A difference in survival of males in both treatment groups compared to the control group.
- C. An increased incidence of pyelitis and liver necrosis in Q7-2159A implanted males and an increased incidence of lung edema in MDF-0193 implanted males.

A question has arisen regarding the possibility that silicone gel might provide a "sink" or depot for endogenous lipophilic hormones and that this could result in a biologically relevant imbalance in the endocrine system.

A data review was performed for the life-time rat study of Q7-2159A appearing as Report Reference 153 with regard to data which might support the premise that implantation of silicone gel produced treatment-related effects on endocrine organs.

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Clinical signs gross pathology and organ weights, and histopathology findings were evaluated. No data were developed which could substantiate the premise that life-time exposure to S.Q. implanted silicone gel affected the endocrine system. Based upon specific endocrine related data, there is no information that suggests that silicone gel affects the structure or function of the endocrine system or that silicone gel may act as a "sink" for steroidal or other hormones thereby causing adverse effects in the endocrine system.

As additional support for the conclusion that gel does not induce endocrine imbalance consider that silicone gel implanted

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subcutaneously in the rabbit 6 weeks before insemination does not cause fetal toxicity or teratogenicity (Report Reference 129). Certainly, effects on reproductive performance, gestation and fetal development constitute a sensitive indicator of possible imbalances in endocrine function.

3. LIFE-TIME RAT STUDY OF Q7-2159A (Report Reference 154) -

This rat life-time study of silicone gel Q7-2159A was initiated in January, 1991 for the purpose of evaluating the chemical carcinogenic potential of a silicone gel when solid-state tumorigenesis is a confounding factor. This study includes the subcutaneous implantation of Q7-2159A in groups of 100 female rats at 0.8, 3.6 or 16.0 ml total dose per animal. Control groups were implanted with U.S.P. polyethylene. All observations to date are consistent with the life-course of Sprague-Dawley rats and are generally unremarkable.

4. TWO-YEAR STUDIES WITH MINIATURE MAMMARY IMPLANTS IN DOGS (Report Reference 155) -

In this 1970 dog study 2 miniature mammary implants were implanted in a submammary position in each of 4 beagles. These dogs also received multiple samples of silicone elastomer implanted subcutaneously. At 6 months after implantation the miniature mammary implants were encapsulated with fibrous connective tissue characteristic of a mild, stable foreign body reaction. There was no evidence of a chronic inflammatory reaction. One dog was necropsied at 2 years. In the animal there was stable encapsulation and evidence of a persistent chronic inflammation.

On the basis of these subchronic and chronic studies of various silicone gels it is concluded that:

1. Silicone gels are associated with a typical foreign body reaction consisting of initial acute inflammatory infiltration superseded by chronic inflammation (macrophages and multinucleated giant cells) transitioning to fibroblastic activity and connective tissue encapsulation. There may be a persistent sparse inflammation in some animals.

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2. So-called solid-state tumorigenesis (SST) occurs in the presence of silicone gels. That is, sarcomas occur at the implantation site and are derived from the connective tissue capsule. The response occurs in males and females. There is no present evidence of chemical carcinogenesis in contrast to the epigenetic SST mechanism.
3. In the most recently completed life-time rat study of silicone gels the following non-neoplastic events were observed at a statistically significant incidence.
 - A. Reduced survival in male rats.
 - B. Increased pyelitis and liver necrosis in male rats.
 - C. Increased lung edema in female rats.

The biological relevance of these findings is unknown although such effects have not been observed in human experience.

ABSORPTION/DISTRIBUTION/METABOLISM/EXCRETION (ADME):

There are 2 ADME studies that have been done with silicone gel.

1. **DISTRIBUTION AND ELIMINATION OF SILICONE GELS IN RATS AND RHESUS MONKEYS (Report Reference 156) -**

One male rhesus monkey was injected subcutaneously with about 5 gm/kg body weight of each silicone gel formulation (3 animals for the "responsive" gel). The gels included the original so-called "stiff" gel (MDF-0193), the new "responsive" gel (Q7-2159A), a high fluid content gel and a low cross-linker gel. Excreta were collected from these animals for 14 days followed by sacrifice on day 15 for tissue collection. Total elemental silicon and organic solvent extractable silicon was measured by emission spectroscopy. One monkey served as an untreated control. An eighth monkey received several subcutaneous injections of the low cross-linker gel for examination of bulk gel movement.

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SUMMARY AND CONCLUSIONS

In order to verify the biological safety of Mentor's Gel-Filled Mammary Prostheses for their intended use, Mentor Corporation has conducted extensive pre-clinical safety testing on the sterile finished devices, raw materials, and components used to make these devices. The biological/toxicological testing is fully described in this current submission for Mentor's Gel-Filled Mammary Prosthesis PMA. Raw materials testing was performed to initially verify that any chosen material had no obvious biological incompatibilities. Finished device testing was conducted to verify that the materials in the device and the configuration of the device (after its full manufacturing and sterilization processing) had no biological incompatibilities. Where it was not practical or possible to test the whole device, component testing was used in place of the full product testing.

The August 13, 2001 document "Guidance for Saline, Silicone Gel, and Alternative Breast Implants; Final Guidance for Industry" from FDA detailed the important toxicological issues to be addressed by pre-clinical testing of these devices. Those issues are the following:

- Pharmacokinetic studies
- Biocompatibility testing
 - Cytotoxicity
 - Short and intermediate-term implantation tests
 - Acute systemic toxicity
 - Hemocompatibility
 - Immunotoxicity
 - Reproductive toxicity and teratogenicity (two generation study)
 - Genotoxicity
 - Carcinogenicity (including sub-chronic and chronic toxicity)

The following discussion details how Mentor's broad battery of biological/toxicological testing together with a review of information from available literature, addresses each of these issues and confirms the biological safety of all three Mentor Gel-Filled Mammary Prostheses product lines (Moderate Profile, Moderate **Plus**, and High Profile).

It should be noted that most of the testing described in this submission has been conducted in accordance with the available established guidelines and standards (e.g., Tripartite, USP, ASTM, ISO), and has included testing of device extracts and/or device materials, depending on the protocol.

Pharmacokinetic Studies:

As part of a toxicological evaluation, pharmacokinetics can provide insight into mechanisms of toxicity of chemicals and can serve as an important predictive tool for the extrapolation of any toxicity observed in animals to the likely risks for humans (*FDA Redbook II* 1993, *Hayes Principles and Methods of Toxicology* 2000). However, in the absence of any significant toxicity findings in adequate toxicity studies, and especially in cases where the exposure is

internal (i.e., rates of absorption from the gastrointestinal tract or lungs are not an issue), pharmacokinetic data are less useful. Dr. Young (NCTR, FDA) at the 1991 Conference on Silicone in Medical Devices, noted that "pharmacokinetics as a tool is really of limited usefulness" for materials such as silicone "because of the extremely slow and limited movement of silicone from the initial site of administration." As FDA has acknowledged, pharmacokinetic studies of elastomer shell materials would in most, if not all cases, be entirely impractical, and there are no validated methods to conduct such a studies.

Nevertheless, given that Gel-Filled Mammary Prostheses consist of silicone elastomer shells and silicone gel filler encased within the shell, the safety issues (raised by FDA and others) that might potentially relate to pharmacokinetics can be divided into three categories and addressed by existing data. The first issue is whether minute amounts of residual low molecular weight materials used in the synthesis of the elastomer might be released into the surrounding tissue; second, whether particles of silicone elastomer might be abraded from the shell and released into surrounding tissue; and third, whether silicone gel might become available either via rupture of the silicone elastomer shell or diffusion through an intact silicone elastomer shell. These three potential issues will be discussed separately.

Potential Release of Low Molecular Weight Materials: As is generally the case for polymers, the potential exists for release into surrounding tissue of minute amounts of residual low molecular weight materials that are used in the synthesis of the elastomer materials for the prostheses. Detailed analytical chemistry results, including quantitation and identification of potentially extractable species, will be provided in the subsequent chemistry data submission of this PMA. The review of available toxicity data, on those chemicals for which approximate levels in the final device are currently known or may be estimated, indicates that most if not all are below the documented levels producing effects in humans or laboratory animals. As noted above, confirmatory data regarding the levels of extractable materials present in Gel-Filled Mammary Prostheses will be provided in the chemistry data PMA submission to be submitted in the near future. Furthermore, short- and long-term toxicity testing that has been performed on components or finished sterilized devices has already shown a lack of toxicity from the presence of these extractable materials.

Potential Release of Silicone Elastomer Particles: In the two-year rat chronic toxicity/carcinogenicity bioassay of Gel-Filled Mammary Prosthesis materials, pulverized shell elastomer was used as a test material. Implantation of these elastomer particles both exaggerated the dose of potentially extractable materials (by vastly increasing the exposed surface area) and provided an evaluation of any potential systemic toxicity that might be produced by the silicone particles. No evidence of systemic toxicity was observed in the study. These results are consistent with the published reports indicating a lack of any clinically significant effects of silicone particles in patients with mammary prostheses or similar silicone implants (i.e., genitourinary prostheses).

Potential Release of Gel From a Ruptured Shell or Via Diffusion Through the Shell:
Mentor has used [REDACTED] silicone gel to date - [REDACTED]

differences were found in interferon-gamma levels among [REDACTED] treatment groups (with multiple doses of and without shell implants), nor were any other tested serum cytokine levels detected in any group.

Studies have shown that silicone gel can provoke a cell-mediated response only when administered under extraordinary conditions. One study has shown that silicone gel can act as an adjuvant in rats while others have demonstrated that only when silicone gel is mixed or emulsified with the antibody can the gel act as an adjuvant. In the clinical setting, it is unlikely that this mixing of gel and antigen in an emulsion-like form would occur. Finally, none of these studies has suggested that silicone elastomer has adjuvant activity or any ability to stimulate the immune system. In a study by Dr. N. Rose, silicone elastomer particles containing some of the materials found in Mentor's Gel-filled Mammary Prosthesis shells were reported to have no apparent adjuvant effect in rats, using the same test model as that in which the gel adjuvant effect was observed.

Teratology and Reproductive Toxicity Studies: Mentor has conducted a two generation study in rats to assess the teratogenic and reproductive toxicity potential of Mentor's Gel-filled Mammary Prosthesis shell. In order to exaggerate the dose of potentially extractable materials the elastomeric test material was pulverized prior to implantation, thus vastly increasing the exposed surface area. The findings of this study indicated that, compared to the controls, pulverized patched and/or valved silicone elastomer mammary prosthetic shells did not cause reproductive or teratogenic effects when implanted subcutaneously in female rats in two consecutive generations. Dow Corning has conducted reproduction and teratology studies on silicone gel Q7-2159A (which is equivalent to the silicone gels Mentor has used historically and currently). The results indicated that there were no treatment-related effects on F₀ parental general conditions and reproductive performance, F₁ neonatal viability, or growth in a rat reproduction study and no maternal or developmental effects in a rabbit developmental toxicity study. These results are consistent with published data in showing that silicone materials are neither reproductive toxicants nor teratogens in animals. Finally, [REDACTED] contains a teratology study on their gel (which has been determined to be equivalent to [REDACTED]). No teratogenic effects attributable to the implanted material were seen in this rat study.

Genotoxicity Testing: Mentor has conducted mutagenicity testing on finished sterilized Gel-filled Mammary Prostheses. Three assays were conducted with extracts of Gel-filled Mammary Prostheses (cut open to expose the shell and gel) including: bacterial mutagenicity (Ames), DNA damage (unscheduled DNA synthesis in mammalian cells), and chromosome aberrations (chromosome aberrations in Chinese Hamster Ovary cells). The results from these assays were universally negative, indicating a lack of genotoxic activity. These findings are consistent with genotoxicity investigations found in the literature.

Acute and Subchronic Toxicity Studies: Mentor and its material vendors have completed a broad battery of acute toxicity testing on raw materials, components, and finished sterilized devices. Such testing has included irritation tests, sensitization tests, cytotoxicity, acute systemic toxicity, blood compatibility, pyrogenicity (material-mediated), and implantation tests. The results from these studies have consistently demonstrated a lack of acute toxicity.

Chronic Toxicity and Carcinogenicity Testing: Mentor has conducted a two-year bioassay in rats to evaluate the chronic toxicity and carcinogenicity potential of Mentor's textured and smooth Gel-filled Mammary Prostheses shell. In order to exaggerate the dose of potentially extractable materials the elastomeric test material was pulverized prior to implantation, thus vastly increasing the exposed surface area. The materials associated with these Gel-filled Mammary Prosthesis shells did not produce chronic systemic toxicity. Various degrees of inflammatory responses were observed at the site of implantation. Carcinogenicity was limited to the development of implant site related sarcomas, primarily fibrosarcomas, and their subsequent effects. The presence of fibrosarcomas at the implant sites represents a neoplastic response commonly observed in rodents following the subcutaneous implantation of a broad range of foreign materials. As discussed in the literature review section on the potential carcinogenicity of silicone, solid-state carcinogenesis observed in rodents is not considered relevant to human risk. Furthermore, strong epidemiological evidence from at least two large studies involving women with mammary prostheses indicate that there is no increased risk of breast cancer associated with these devices.

Chronic toxicity testing of Mentor's previous vendor silicone gels can be found in [REDACTED] and in the case of Dow Corning gel, publicly available documents detailing their testing. Both Mentor's current gel [REDACTED] have been shown to be not substantially different from Dow Corning's gel based on testing of the raw materials and finished products. [REDACTED] contains a two year chronic toxicity/carcinogenicity test in rats in which no evidence of systemic toxicity attributable to the implant material was found. The only abnormal finding was the occurrence of implant site fibrosarcomas and their subsequent effects. Dow Corning has performed three chronic toxicity studies in rats on Q7-2159A gel. While some of the data in earlier studies produced equivocal results, the most recent test involving 700 animals definitively demonstrates that mammary silicone gel does not contain a chemical carcinogen. All three studies encountered the occurrence of solid state tumorigenesis as has been noted in studies of silicone and other materials.

As a result of the chronic toxicity/carcinogenicity testing on Mentor's Gel-filled Mammary Prosthesis shells and silicone gel, and considering the data from publicly available literature, Mentor believes that the components of sterile gel-filled mammary prostheses do not cause adverse systemic effects (aside from expected solid state tumors not considered relevant to humans) when implanted in rats during chronic toxicity testing.

Overall Conclusion: Taken together, the results from Mentor's broad battery of biological/toxicological testing, along with a review of information from available literature, confirms the biological safety of Mentor's Gel-filled Mammary Prostheses for their intended use.